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A SUBCHRONIC INHALATION STUDY OF FISCHER 344 RATS EXPOSED TO 0, 0.4, 1.4 or 4.0 ppm ACROLEIN

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for
THE NATIONAL TOXICOLOGY PROGRAM
under
INTERAGENCY AGREEMENT NUMBER
222-Y01-ES-9-0043

October 1981

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Conducted at The Medical Department

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ACKNOWLEDGEMENTS

The authors wish to thank William Maston and Peter Bonti for chamber operation and animal care. The technical assistance of James Lehmann and Elizabeth Jellett for pulmonary function testing; Martine O'Connor for necropsy; Max Schmaeler for biochemistry; Michael Torelli for reproductive studies; and Thomas Vogt for cytology is most appreciated. Thanks are extended to Charles Bores, computer programmer and Patricia Hu, biometrician. We thank Dr. Robert Wehner for the follow-up pathology observations presented in Appendix H. Our appreciation is also extended to Jayne Cutt for secretarial services. Special thanks are extended to Dr. Sonja Haber for her review of this document.

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LIST OF ABBREVIATIONS

ANOVA: analysis of variance

atm: atmosphere

ATPD: ambient temperature pressure dry

BMDP: Biomedical Program

BrdUrd: bromodeoxyuridine

BTPS: body temperature pressure standard.

C_{DYN}: dynamic compliance

$DLCO_{rb}$: diffusing capacity of the lung for CO measured by rebreathing technique

$DLCO_{sb}$: diffusing capacity of the lung for CO measured by a single-breath technique

EFR_x: expiratory flow rate at x% vital capacity

ΔEFR_{25}: difference in the flow at 25% vital capacity above or below that volume estimated by a chord slope drawn from EFR_{50} to EFR_0.

EKG: electrocardiogram

EPL: Experimental Pathology Laboratories, Inc.

ERV: expiratory reserve volume

f: frequency

FRC: functional residual capacity

FRC_b: functional residual capacity determined by Boyle's law

FRC_d: functional residual capacity determined by dilution

HR: heart rate

IC: inspiratory capacity

IRV: inspiratory reserve volume

M_0: total area under the N_2 washout curve for 50 breaths $M_0 = \sum_{j=1}^{50} X_j$

M_1: $\sum_{j=1}^{50} j \cdot X_j$

M_2: $\sum_{j=1}^{50} j^2 \cdot X_j$

MEFV: maximum expiratory flow volume

MFSR: maximum flow static recoil

ns: not significant

p: probability

P: pressure

P_{ao}: airway pressure

PBS: phosphate-buffered saline

P_e: esophageal pressure

PEF: peak expiratory flow

PHA-P: phytohemagglutinin-P

P_L: transpulmonary pressure

ppm: parts per million

P_{st}: static pressure

QSC: quasi-static compliance

QSC_{cs}: quasi-static compliance determined by chord slope

QSC_{ss}: quasi-static compliance determined by steep slope

R_L: pulmonary resistance

R_{us}: upstream airway resistance

RV: residual volume

SCE: sister chromatid exchange

SPF: specific pathogen free

TLC: total lung capacity

TLC_d: total lung capacity determined by dilution

V: quasi-static volume

$\dot{V}$: airflow

$\dot{V}_E$: minute volume

VC: vital capacity

V_T: tidal volume

V_{TG}: trapped gas

SUMMARY

Fischer 344 rats were exposed to 0.0, 0.4, 1.4, or 4.0 ppm acrolein for 62 days. The major objective of the study was to relate the results of a series of pulmonary function tests to biochemical and pathological alterations observed in the lung. Cytological and reproductive potential endpoints were also assessed after acrolein exposure.

Rats were exposed to acrolein for 6 hours/day, 5 days/week for 62 days. Mortality was observed only in the 4.0 ppm chamber where 32 of 57 exposed males died; however, none of the 8 exposed females died. Most of the mortality occurred within the first 10 exposure days. Histologic examination indicated that the animals died of acute bronchopneumonia. The surviving males and females exposed to 4.0 ppm acrolein gained weight at a significantly slower rate than control animals. The growth of both sexes in the 0.4 and 1.4 ppm groups was similar to that of their respective controls.

Histopathologic examination of animals after 62 days of exposure revealed bronchiolar epithelial necrosis and sloughing, bronchiolar edema with macrophages, and focal pulmonary edema in the 4.0 ppm group. These lesions were, in some cases, associated with edema of the trachea and peribronchial lymph nodes, and acute rhinitis which indicated an upper respiratory tract effect of acrolein. Of particular interest was the variability of response between rats in the 4.0 ppm group, some not affected at all while others were moderately affected. Intragroup variability in toxicity was also apparent in the 1.4 ppm exposure group where only 3 of 31 animals examined had lesions directly related to acrolein exposure. Extra respiratory organs appeared unaffected.

Pulmonary physiology tests revealed a substantial decrement in the pulmonary function of rats exposed to 4.0 ppm acrolein. The pattern observed suggested obstructive lung disease with virtually every static and dynamic

parameter significantly affected. A depressed flow-volume effort, a leftward shift of the quasi-static compliance curve, and an enlarged lung volume were all consistent with a classical obstructive lesion. While the pulmonary function of the 4.0 ppm group suggested an obstructive lesion, the data from the 0.4 ppm group indicated a restrictive lung lesion. The parameters of spontaneous breathing, and the divisions of lung volume were unremarkable in the low dose group, however, the flow volume maneuver exhibited "supra-normal" flows. This could have resulted from more rigid airways without parenchymal damage. The pulmonary function of the 1.4 ppm exposure group was between that of the low and high dose groups and was nearly identical to that of control animals. These data suggested the development of two functional lesions exhibiting opposing effects on the pulmonary function-measurements. It should be noted that the lesion manifesting itself in animals exposed to 0.4 ppm acrolein was not morphologically evident upon histopathologic examination.

The lungs of rats exposed to 4.0 ppm acrolein were heavier than those of the larger control rats. A 20% increase in the dry weight was accompanied by a 1.5% increase in water content. This increased dry weight and the absence of a significant change in the amount of DNA and protein per unit dry weight indicated that the increased lung weight of this group was at least in part due to increased cellularity. Lung connective tissue content increased as a result of acrolein exposure. Elastin concentration in the lungs of the 4.0 ppm animals was twice that of control animals. Elastin content of the lungs from the 0.4 and the 1.4 ppm exposed animals was similar to that of the control group. Hydroxyproline (an index of collagen content) concentrations increased significantly in both the intermediate and high dose groups. When based on dry weight the hydroxyproline concentrations of the 1.4 and 4.0 ppm groups were 111% and 133%, respectively, of control levels.

The cytological endpoints assessed included sister chromatid exchanges and cell proliferation kinetics in bone marrow cells and peripheral blood lymphocytes. The incidence of chromosomal aberrations was also examined in peripheral blood lymphocytes. No statistically significant changes were found among these parameters.

The sperm of exposed animals was examined for morphologic abnormalities but none were evident and the percentage of morphologically abnormal sperm was similar in control and acrolein exposed animals. The reproductive potential of male and female Fischer 344 rats was unaffected by acrolein exposure.

INTRODUCTION

In recent years the technology has been developed to measure several indices of pulmonary function in rodents. These include: static lung volumes, static and dynamic lung properties, assessment of diffusion capacity, and the distribution of ventilation. Standard toxicity evaluations of airborne materials rarely include assessment of pulmonary function, in part because the applicability of these measurements in the assessment of pulmonary toxicity remains to be demonstrated. In the past, respiratory function tests were generally not as sensitive an index of pulmonary damage as morphologic examination. However, it should be determined whether the recent developments have increased the sensitivity of rodent pulmonary tests. The relative sensitivity of the two indices should be examined so that the cost-benefit ratio of incorporating pulmonary assessment into inhalation toxicology protocols can be evaluated.

The major purpose of this study was to compare the morphological, biochemical, and functional changes induced by exposure to acrolein. This aliphatic aldehyde is a strong cytotoxic and ciliostatic agent (1-3), and its irritating effect on mucus membranes and its acute inhalation toxicity properties have been reported (4-7). Acrolein causes broncho-constriction in guinea pigs (8) and reduced pulmonary compliance in mice (9); therefore, it was an appropriate agent for these studies which were designed to relate pulmonary function to associated pathology and changes in structural components of the lung.

The exposure chambers employed in these studies housed more animals than needed for pathological, physiological, and biochemical assessment; therefore, several other endpoints were investigated. The genetic effects of acrolein exposure were assessed with an assortment of interrelated

cytogenetic endpoints. Acrolein has been reported to cause impairment of DNA replication *in vitro* (10). In addition, sperm morphology studies were conducted and the reproductive potential of both male and female exposed rats was assessed.

MATERIALS AND METHODS

Animal Procedures and Exposures

The Fischer 344 rats used in this study were obtained from Charles River Laboratories, Inc. (Kingston, N.Y.). The animals were received in two shipments and housed in our SPF (specific pathogen free) facility for approximately four weeks before exposure. During this quarantine period, 10/200 and 9/185 rats from the first and second shipments, respectively, were sent to AnMed Laboratories, Inc. (New Hyde Park, N.Y.) for health assessment. This service included: determination of serum viral antibody status (Sendai Virus, Pneumonia Virus of mice, Reo Virus Type 3, Theiler's Virus, Kilham's Rat Virus, Lymphocytic Chorimeningitis, and Rat Chronona Virus); culture of nasoturbinate washings to check for respiratory bacterial pathogens and mycoplasma; oropharyngeal swab for detection of pseudomonas and klebsiella; preparation of fecal samples for bacterial pathogen and parasite detection; preparation of ideal wet mounts for protozoans; inspection of the colon for helminths and of the bladder for Trichosomoides crossicauda; and scanning of the pelt for ectoparasites. Slides for histopathological examination were prepared from the lung, liver, kidney, ileum, spleen, and thymus. Citrobacter freundii was found in the feces and upper respiratory tract of all animals from both shipments. This organism has not been reported as pathogenic in rats; however, it is associated with colonic hyperplasia and diarrhea in laboratory mice (11). Although C. freundii was an unusual and undesirable finding in these animals, its presence was interpreted as not interfering with the use of these animals in the proposed protocol.

During the holding period the rats were ranked by weight and randomly assigned to a particular exposure group. All of the animals were neck tagged to provide permanent identification. The animals were individually

housed in stainless steel wire mesh cages and provided a standard laboratory diet (Purina Chow) and water ad libitum. A 12-hour-on/12-hour-off light cycle was maintained in the animal room.

Experimental and control animals were placed into the appropriate chambers the night before the initial exposure. Caging and light cycle in the chambers were identical to those in the holding rooms. The cage units (each holding 8 rats, 2 rows of 4) were arranged in 3 tiers with 3 units per tier. Once placed into the chambers, the rats were housed there for 24 hours/day. Water was supplied to the chamber animals ad libitum; however, the food was removed during the daily 6 hour exposure period. Each animal was weighed after the first exposure day and then weekly according to the following schedule: control rats, Mondays; 0.4 ppm rats, Tuesdays, 1.4 ppm rats, Wednesdays; and 4.0 ppm rats, Thursdays.

The animals were briefly examined each day prior to exposure, when the food troughs were removed and clean catch pans were provided, and again when the food troughs were replaced following the exposure period. The animals were also inspected once daily on weekends. When the animals were weighed they were examined more closely and provided a clean cage. The cage packs were rotated through nine positions (3 tiers with 3 units/tier) by moving each pack one position after the weekly weighing.

Rats were exposed to either filtered air, 0.4 ppm, 1.4 ppm, or 4.0 ppm acrolein for six hours/day, five days/week. Each animal was exposed for 62 consecutive days with exceptions only for weekends. Each rat was exposed a minimum of two days the first and final weeks of exposure. In cases where the end-point test procedures were time consuming, the starting dates were staggered while still adhering to the 62 exposure day regime and the minimum number of exposure days per week. With the exception of the rats

designated for cytology studies, rats were placed into SPF animal rooms for six days after the final exposure. Cytological endpoints were assessed the day after the final exposure.

Animals in the chambers were utilized as follows: Twenty-four animals were placed into each of the four chambers for respiratory physiology studies. After pulmonary function testing, these animals were sacrificed and the lungs carefully removed. The right lung of each animal was submitted for biochemical analysis and the left lung was processed for pathological examination (see Pathologic Examination). Eight rats in each chamber were designated for pathology only. Ten animals from each exposure level were designated for various cytological studies. Eight male and eight female rats were exposed for reproductive studies. The high mortality rate among male rats in the 4.0 ppm exposure chamber reduced the sample size of the high dose group in most studies.

Chambers

Exposures were carried out in stainless steel and Lucite chambers. Airflow through the 5 m^3 chambers was 1 m^3/min. Exhaust air from each chamber was passed through a trap containing activated charcoal before being discharged. The relative humidity was continuously monitored by placing a Honeywell (Model 612 x 9-HT) humidity recorder into the control chamber. During the exposure periods, the temperature at several locations in each chamber was monitored with thermocouples wired to a Fluke Datalogger. During nonexposure hours the temperature of the control chamber was recorded on the Honeywell instrument used to record relative humidity.

Acrolein Generation

Gaseous acrolein (for chemical and physical characteristics see Appendix A) was purchased as an analyzed 1000 ppm mixture in nitrogen (Union Carbide Corporation and Scientific Gas Products, Inc.). The gas mixture regulated to 6 psig was delivered to a glass reservoir from which it was metered via valved rotometers into the air supply lines of the exposure chambers. The glass reservoir was also fitted with a pressure gauge and another valved rotometer by which excess gas was bled off. This discharge system provided greater independent control of the rotometers for the individual chambers. Excess gas released from the reservoir was passed through an activated charcoal trap before discharge into the chamber exhaust system.

Monitoring of Acrolein Concentrations

The acrolein concentration of each chamber was automatically monitored for five minutes every half hour with a Miran infrared analyzer (Model 80A, Foxboro). The absorbance readings were converted to ppm values using linear regression calibration plots established with chemical analysis techniques for acrolein (12) (Appendix B). The acrolein concentration in each chamber was chemically determined at least bi-weekly (with only two exceptions, Appendix C). Miran absorbance data corresponding to the three most recent chemical determinations for each of the chambers were used to establish a current linear regression plot. The chambers were automatically sampled and the data recorded hourly. After the first 1/2 hour of operation, the data were used to adjust chamber concentrations and calculate mean daily concentrations.

Necropsy of Moribund and Dead Animals

Rats found in a moribund condition were killed with a lethal dose of

pentabarbitol and exsanguinated via the descending aorta. The lungs of killed and dead animals were removed, the heart, thymus, and excess tissue were trimmed away and the lungs were weighed. The lungs were then fixed with 2.5% glutaraldehyde in Sorenson's buffer via the trachea at 25 cm water pressure for 30 minutes. After tracheal fixation, the lungs remained in glutaraldehyde fixative for a minimum of 24 and a maximum of 72 hours. The lungs were then rinsed with four changes of Sorenson's buffer over a 24 hour period and stored in this buffer until prepared for sectioning by Experimental Pathology Laboratories, Inc. (EPL).

Respiratory Physiology

A series of pulmonary function tests were performed on each animal designated for respiratory assessment. A constant volume plethysmograph (2.2 liter), maintained isothermal with an attached 16 liter reservoir bottle filled with copper mesh, was used for all measurements. This reservoir was insulated on all sides with foam rubber. In addition to the removable faceplate needed to insert the animals, the plethysmograph was equipped with several ports for the passage of EKG and transducer leads (Figure 1).

Lung volume changes were measured as proportional pressure changes using a high frequency response differential pressure transducer (Setra System 239: $\pm$ 0.01 atm) referenced to a 20 liter bottle filled with copper mesh. This transducer was embedded directly into the wall of the plethysmograph to minimize frequency damping. Intra-thoracic pressure was measured with a differential pressure transducer (Sanborn 268B: $\pm$ 40 mm Hg) via a water-filled catheter (PE-160) inserted into the esophagus of the rat to a depth of 10 cm from the upper incisor teeth. From the side of the 4 mm breathing port of the plethysmograph, a second water-filled catheter (Pentube 1, AWG #15) was connected to the reference side of the 268B transducer. The

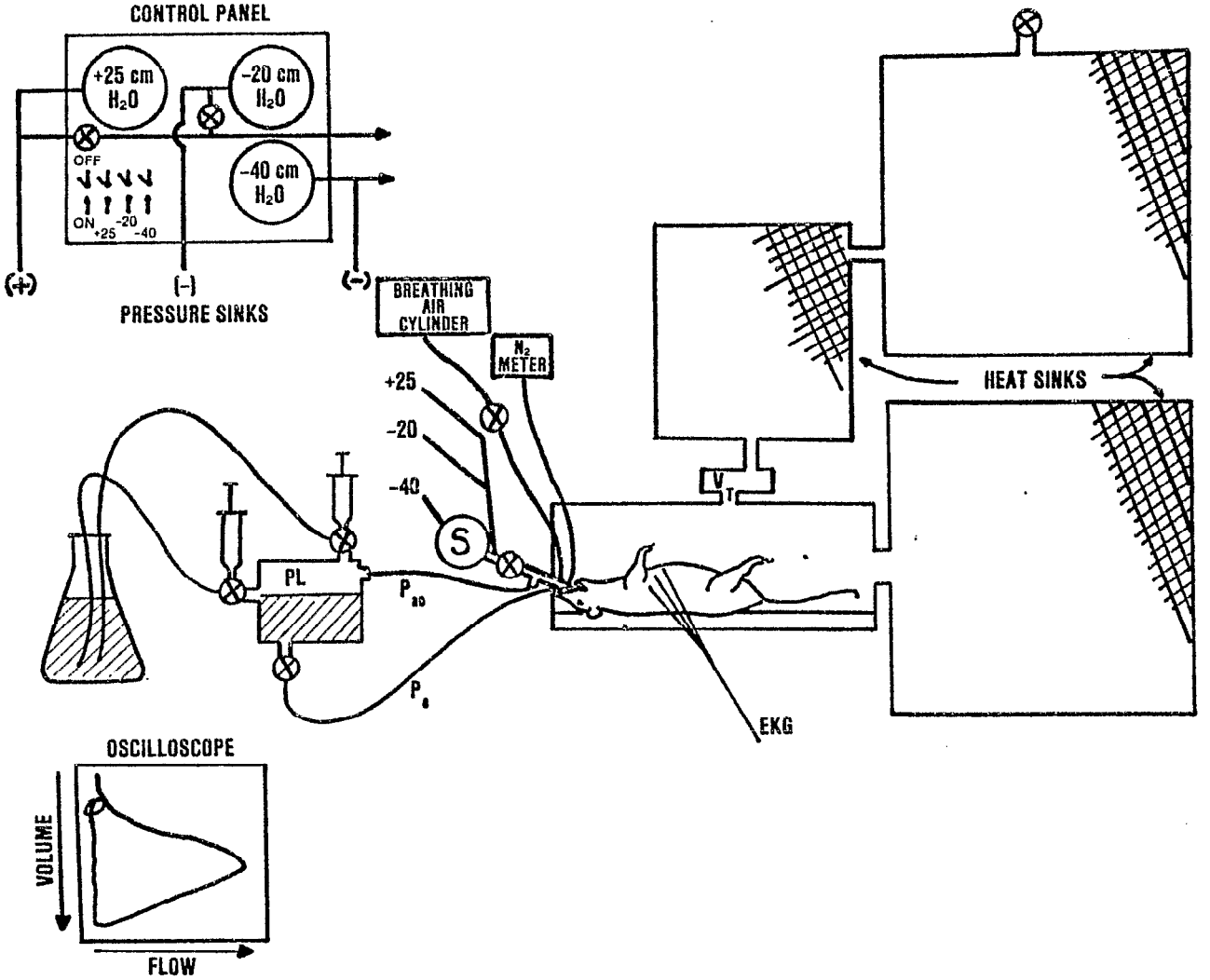


Figure 1: Schematic diagram of the plethysmograph and associated instrumentation to assess small rodent pulmonary function.

electronic subtraction of the esophageal pressure (P_e) from airway pressure (P_{ao}) provided the transpulmonary pressure (P_L) or driving pressure of the lungs. Prior to animal testing, esophageal and airway catheter lengths were adjusted to ensure a constant phase relationship of transpulmonary pressure and plethysmographic pressure (calibrated as volume) to a frequency of 6 Hz using a piston pump (1 cc displacement).

When specific breathing maneuvers were not being imposed, tidal volume (V_T), frequency of breathing (f), P_L, air flow (V) as derived from V_T, pulmonary resistance (R_L), and dynamic compliance (C_{DYN}) were recorded. Signal conditioning was achieved using HP-8805C carrier preamplifiers for V_T and P_L. The R_L and C_{DYN} were calculated by an analog computer (HP-8816A Respiratory Analyzer) according to the method of Mead and Whittenberger (13). Airflow, as derived by the computer module, and C_{DYN} were conditioned through a HP-8802A medium gain preamplifier. Three-lead EKGs (equivalent two lead configuration) were obtained from each animal just prior to insertion into the plethysmograph. The lead (needle) configurations formed a triangle across the animal's chest. The ground lead was attached at the base of the left front leg, the negative pole was located at the base of the right front leg and the positive pole was positioned centralaterally just below the animal's seventh rib. A second configuration with the negative lead positioned at the regional apex of the heart was also used. Heart rate, standard intervals of cardiac electrical activity, and wave forms were evaluated from these tracings. An eight-channel recorder (Gould, Brush 2800) was used for all of the above parameters.

Each animal was anesthetized with 75 mg/kg pentabarbitol (Nembutal). Controlled anesthesia was achieved by injecting 67% of the total dose followed by the remaining 33% after the loss of righting reflex. This resulted in a relatively stable level of anesthesia for a period of

approximately two hours, sufficient time for assessment and subsequent sacrifice.

Each rat was placed in the plethysmograph in a supine position. A cannula, molded from teflon shrink tube, was transorally inserted into the trachea, effectively by-passing the effect of the nose on all of the parameters quantified in these otherwise obligate nasal breathers. Approximately 1 cm from the proximal tip of the cannula, a shoulder was molded to ensure an airtight seal with the glottis upon insertion. The total dead space of the cannula, including all valving to the glottis insert, was measured manometrically and adjusted to BTPS (body temperature pressure saturated). The volumes of the tracheal cannulas used ranged from 1.55 to 1.90 cm^3. The "effective" dead space from the mouth opening to the distal end of the breathing port was 0.71 cm^3. To offset the effect of this latter dead space on the parameters of spontaneous breathing, a bias flow of breathing air (approximately 400 cm^3/min) was introduced into the tracheal cannula through a side port to maintain fresh air in that space. The bias flow was curtailed during all other measurements.

The rat was allowed to stabilize within the system for approximately 10 to 15 minutes. This period was determined by the stability of spontaneous breathing parameters, R_L and C_{DYN}. When these tracings had satisfactorily stabilized, their average values over a 0.5 minute period were noted. Subsequently, a series of ventilatory maneuvers were performed on each animal to assess the following: divisions of lung volume, quasi-static compliance (QSC), multibreath N_2 washout, and characterization of the maximum expiratory flow volume (MEFV) maneuver. The TLC and RV were defined as those lung volumes corresponding to a transpulmonary pressure of +25 cm H_2O and -20 cm H_2O, respectively. Inflation and deflation of the lungs, from end-expiration (the end of a normal tidal breath), were achieved through the use

of a large-volume, constant-pressure reservoir controlled by a solenoid valve. Quasi-static volume (V)/pressure (P_L) relationships were determined in a similar manner, but were dictated by a defined inspiration (∿3 ml/sec) to TLC and a slow deflation (∿3 ml/sec) to RV. The volume-pressure curves were recorded on an X-Y plotter (HP-7045A). Quasi-static compliance was measured both as the tangent slope to the steepest portion of the curve (QSC_{ss}) above the functional residual capacity (FRC) and as the chord slope (QSC_{cs}) from 0 to 10 cm H_2O P_L. The pressure span for computation of the chord slope was chosen as the typical lower and upper limits of tidal P_L.

The FRC was measured by neon dilution as described by Takezawa et al. (14) and the Boyle's law technique (15). The "standard" gas used in the dilution measurements consisted of 0.532% Ne, 0.497% CO, and 22.01% O_2 in N_2. The volume injected was equal to the plethysmographically determined vital capacity (VC) adjusted to ATPD (ambient temperature pressure dry). From RV, a volume equal to VC (ATPD) was injected from a syringe through a three-way valve. The lungs were then ventilated ten times in approximately ten seconds with this syringe using a stroke volume of approximately 75% of the VC. The component gases of the final VC-volume withdrawn were quantitated on a gas chromatograph (Carle Basis GC 8700). The proportional dilution NE and the VC (BTPS) were used to calculate the TLC_d (TLC by dilution). In conjunction with the measured expiratory CO, it was possible to calculate a "rebreathing" diffusing capacity for CO ($DLCO_{rb}$). Adjusting for equipment dead space and subtracting the measured inspiratory capacity provided the FRC_d (by dilution). The FRC_b (Boyle's law) was determined by occluding the airway at end expiration and comparing ΔP_{ao} (airway) to ΔV with each inspiratory effort. Calculation of $VP = V'P'$ corrected for dead space yielded FRC_b. These calculations were done on-line by an HP-9825 desk-top computer programmed for breath by breath calculation of the FRC_b. Both

measures of FRC (BTPS) represent the resting lung volume, up to the entry of the trachea into the naso-pharynx. The BTPS correction was based on the ambient barometric pressure and the measured body temperature ($\sim 34^{o}C$) of the rat at the time of the specific test.

Diffusing capacity for CO was measured by both a rebreathing and a single breath technique. The rebreathing technique was used to estimate a dilution TLC as described above. The equilibrated alveolar gas concentrations and the time from inspiration (gas injection) to the final expiration (expirate collection) was used in the Krogh (16) calculation ($DLCO_{rb}$). The single breath estimation was determined by the method of Takezawa et al. (14). The injection of a VC volume of standard gas, corrected to ATPD, was held for 8 seconds. At that time 50% of the gas was withdrawn and discarded as mixed dead space and some alveolar gas. The second half of the expirate was assumed to represent alveolar gas. Using the duration of breath hold (10 seconds), the CO uptake and Ne dilution could be used to calculate the $DLCO_{sb}$.

Multibreath N_2 washout was measured by sampling end-expiratory (alveolar) gas directly in the tracheal tube while the animal was breathing 100% O_2 which flowed by the tracheal tube opening at approximately 400 cm^3/min. A total of 50 breaths were sampled for each animal. The natural log of the end-expiratory N_2 concentration was plotted against breath number or dilution value $\frac{V_T \cdot \text{breath \#}}{FRC_d}$ by the HP-9825 computer from data collected on-line during the maneuver. Moment analyses was then used to assess the degree of ventilatory inhomogeneity.

The MEFV curve was an imposed expiratory maneuver. After slow inflation to TLC, a volume held for approximately three seconds, a pressure sink of -40 cm H_2O was exposed to the tracheal port of the plethysmograph by

activating a wide bore solenoid valve (Skinner Valve - V53DB2VAC2, 1/4"-3/32" orifice). The tubing from the sink to the valve, as well as between the valve and tracheal port, was as large and rigid as practically possible. With closed vials used to represent body mass and 10 cc of air injected into the closed plethysmograph, the time to peak flow for the system with the tracheal tube in place was 50 msec. For each animal, peak expiratory flow (PEF), expiratory flow at 50, 25, and 10% VC (EFR_{50}, EFR_{25}, and EFR_{10}, respectively), and the percent expired VC at PEF were recorded. The ΔEFR_{25} was measured as the difference in flow at 25% VC above or below that volume estimated by a chord slope drawn from EFR_{50} to EFR_0. A positive ΔEFR_{25} is a measure of the degree of convexity (away from the volume axis) of the effort independent portion of the MEFV curve and conversely, a negative ΔEFR_{25} is a measure of curve concavity (toward the volume axis).

Using the MEFV and quasi-static compliance data, maximum-flow static recoil (MFSR) curves were derived for the determination of "upstream" airway resistance during the MEFV maneuver. The upstream airway resistance (R_{us}) of each animal was calculated as the static pressure (P_{st} divided by $\dot{V}$) at 45% of its lung volume. The existence of airway obstruction and/or loss of tissue elasticity as the potential cause of the decreased flow thereby could be deduced.

Pathological Examination

Animals from each chamber designated for pathological examination were anesthetized with Nembutal and exsanguinated via the descending aorta. The thorax was opened and the heart and lungs were removed intact. The trachea was detached at the larynx and the thymus, heart, lymph nodes, epicardial fat, and esophagus were carefully removed from the respiratory tissue. The lungs were patted dry and weighed with the trachea still attached. The

lungs were then infused with 2.5% glutaraldehyde in Sorenson's buffer at 25 cm water pressure for 30 minutes. After the infusion period, the left lung of four randomly selected animals from each exposure group was submerged in the glutaraldehyde fixative for 3.5 hours, after which tissue slices were removed for possible future electron microscopy studies. The remaining lungs were placed in 10% buffered formalin immediately after the 30 minute infusion. The remainder of the left lobe from which slices had been removed was also placed in formalin. The following tissues were collected and stored in formalin: eyes, pituitary, thyroid, salivary glands, cervical lymph node, larynx, trachea, thymus, peribronchial lymph node, heart, esophagus, stomach, small intestine, large intestine, liver, pancreas, adrenal, mesenteric lymph node, urinary bladder, gonads, prostate, sternum, rib junction, skeletal muscle, peripheral nerve, skin, spleen, and nasal cavity. All pathological examinations were done under contract by Experimental Pathology Laboratories, Inc. (Herndon, Virginia). Microscopic examination was conducted on hematoxylin and eosin stained sections of lung, peribronchial lymph node, nasal turbinate, brain, kidney, liver, spleen, testes, and heart from eight control, low dose, and intermediate dose animals and three high dose rats.

The left lung of all the animals which completed the pulmonary function regime was submitted for histopathologic examination. This provided pathology, respiratory physiology, and biochemical data on individual animals, and also served to determine whether the respiratory physiology testing regime itself induced pulmonary damage. Numerical values were generated from the histopathology sections by adding up the values which indicated the severity of the pulmonary lesions observed. The scored lesions included: alveolitis, type II cell hyperplasia, alveolar macrophages, bronchiolar epithelial necrosis and sloughing, hemorrhage,

bronchiolar edema and macrophages and chronic pleuritis. These sums were then ranked 1-n, where n is the maximum number of samples in the group being ranked.

Determination of Lung Composition

The right lung of each rat completing pulmonary function tests was weighed, homogenized in water using a Polytron Homogenizer (Brinkman Instruments), and the total volume brought to 10 ml with water. Suitable aliquots were then taken for determination of dry weight, by freeze drying in tared tubes, and for chemical analyses.

Collagen content was determined and reported as total hydroxyproline in the sample. Hydroxyproline was determined by the method of Bergman and Loxley (17) after hydrolysis of the aliquot in 6 N HCl at 105°-110°C in an evacuated tube for 22 hr. Elastin was considered to be the insoluble protein remaining after treatment of an aliquot with 0.1 N NaOH at 98°C for 0.5 hr. It was determined by the method of Naum and Mogan (18) and compared with a sample of bovine ligamentum nuchae elastin (Sigma) as standard. Total protein was determined by the Hartree (19) modification of the Folin-Lowry method. DNA was determined according to Burton (20) after heating a sample in 5% perchloric acid at 90°C for 12 min (conditions found to give the maximum color).

Cytological Methods

On the sixth day following the final exposure rats designated for cytological studies were briefly anesthesized with enflurane, and placed in modified Bollman restrainers (21). Tail veins were cannulated with hubless 23 gauge needles inserted into Clay Adams P.E. tubing attached to 1 ml syringes loaded with isotonic phosphate buffered saline (PBS, pH 7.3). After cannulation, the P.E. 50 tubing was attached to similar tubing

originating at a Watson-Marlow 10-Channel peristaltic pump with auto analyzer tubing (Gamma Enterprises). The pump delivered bromodeoxyuridine (BrdUrd), dissolved in PBS, at a rate of 50 mg/kg body weight/hour (flow rate ∿40 ml/24 hours) for 24 hours. During this time, the animals were provided food and water ad libitum. After 23 hours, the animals were intravenously injected with colcemid (20 mg/250 gms body weight). One hour later the tubing was removed and one hour after that (total elapsed time 25 hours) the animals were sacrificed by barbituate overdose administered intraperitoneally.

Immediately after cessation of breathing, the chest cavity was opened and a sample (∿3 ml) of blood was obtained by cardiac puncture using a heparinized 3 cc syringe and 22 gauge needle. This blood was stored in sterile tubes containing lithium heparin until cultured. Whole blood (.25 ml) was innoculated into 5 ml McCoy's SA medium containing 10% fetal calf serum, (Sigma), and 15 mg/ml phytohemagglutinin-P (PHA-P) (Burroughs Wellcome). Complete cultures were incubated at 38°C for 48 and 72 hours in darkness. Colcemid (.1 mg/ml) (Gibco) was added to each culture four hours before termination. At termination the cultures were centrifuged and the pellet was resuspended in hypotonic KCl (0.075 m) for 15 minutes at room temperature. The cells were then fixed twice in methanol:glacial acetic acid (3:1), and stored at 0°C until slides were processed (22).

Both femurs were removed and the bone marrow rinsed out using PBS. The resulting material was incubated in hypotonic KCl (0.075 m) for 20 min at 37°C, then fixed twice in methanol:glacial acetic acid (3:1), and finally stored at 0°C until processed for slides (21).

Both epididymes were removed and minced in PBS, and the large particles allowed to settle. The resulting supernatant containing sperm was spread onto clean microscope slides and fixed for 10 min in methanol:

glacial acetic acid (3:1) (23,24).

To obtain lung alveolar macrophages, the trachea of each rat was exposed and cannulated with tubing attached to a 5 cc syringe. Five ml of PBS (room temperature) was forced into the lungs and allowed to remain for ∿5 min; it was then removed and placed into a 50 ml centrifuge tube, and another 5 ml volume was forced into the same lungs. When 50 ml PBS had been retrieved from the lungs of each animal, it was centrifuged and the pellet was resuspended in hypotonic KCl (0.075 m) for 25 min at 37°C. The cells were then fixed twice in methanol:glacial acetic acid (3:1) and stored at 0°C until processed for slides (25).

All processed material (with the exception of sperm) was flame-dried on microscope slides, stained with Hoechst 33258 (0.5 mg/ml distilled H_2O) for 20 min, mounted with phosphate:citric acid buffer (pH 7.0), exposed to blacklight fluorescent tubes (∿2.5 cm distance) for 25 min, and then stained with Giemsa (4% Harledo Giemsa and 4% methanol in distilled H_2O) for ∿5.5 min (26). Sperm slides were stained with 0.02% eosin Y for 30 min.

One hundred randomly chosen metaphase cells in each sample were scored for the number of times they had replicated (one, two, or more replications), as distinguished by their BrdUrd staining patterns (27). Where possible, 25 second generation metaphase cells were scored for the number of sister chromatid exchanges (SCEs) (21) and 50 first generation metaphase cells were scored for chromosomal aberrations (28). Finally, 500 sperm were examined from each animal to determine the frequency of morphologically abnormal specimens (22,23).

Reproductive Potential Methods

Six days after the final exposure, eight male rats from the control, 0.4 ppm, and 1.4 ppm acrolein chambers and five from the 4.0 ppm chamber

of the sum of squares between groups to the sum of squares within groups. This is also called the first canonical variate. The second discriminant function, or the second canonical variate, was the function of the highest ratio orthogonal to the first. Similarly, the third function gave the highest ratio conditioned to the orthogonality to the first and second functions. The maximum number of discriminant functions derived was one less than the number of exposure groups involved or equal to the number of discriminant variables in the analysis, whichever was smaller. The corresponding eigenvalue and associated canonical correlations for each function denoted the relative ability of the function to separate groups. When the sign was ignored, the coefficient of the function represented the contribution of its associated variable to the function. If most of the variation among the groups was explained by the first and second canonical variates, the tendency of the groups to form clusters was examined by plotting the first versus the second canonical variables.

To assess the adequacy of the discriminating variables, the original exposed animals were categorized by a series of classification functions, one for each group. These classification functions were derived from the pooled within-group covariance matrix and the centroids for the discriminant variables. These classification functions produced one score for each animal for each respective group and the rat was assigned to the group with the nearest centroid (smallest Mahalanobis D-square). The percent current classification for each group was given by the BMDP 7M procedure (3).

RESULTS

General Toxicology Parameters

Exposure Conditions. The temperature and relative humidity in the chambers was continuously monitored. During the hours of acrolein exposure the temperature was maintained at about 21°C; however, extremes of 18°C and 24°C were recorded. The relative humidity ranged from 50 to 70 percent.

The acrolein concentration in each chamber was automatically measured with a MIRAN 80-A infrared analyzer. The MIRAN absorption readings were converted to ppm values by means of linear regression calibration plots (Figure 2) and these were used to calculate the daily time weighted averages (Table 1). Copies of chamber data sheets from four randomly selected days have been provided in Appendix D.

Animal Mortality. Mortality among male rats exposed to 4.0 ppm acrolein reached 56% (32/57) while none of the female rats in the high dose chamber died. The greatest mortality occurred from the 8th through the 10th day of exposure (Figure 3). Animals were introduced into the chambers on four of the five exposure days of the week; therefore, the high mortality observed on days 8 through 10 was not a reflection of 3-5 consecutive days of acrolein insult during the second week of exposure. Deaths appeared to occur without pattern throughout the week with 3, 4, 6, 4, 8, 5, and 2 deaths on Sunday through Saturday, respectively.

Animal Weights. Both male and female rats in the 4.0 ppm acrolein chamber lost weight during the first 10 exposure days (Figures 4 and 5). Although all of the animals in each exposure group were weighed weekly, only data from the largest sub-groups entering the chambers on a single day were plotted and analyzed (survivors only in the male 4.0 ppm subgroup).

were each housed with two unexposed females for seven days. Eight females from each exposure level were mated with unexposed males (1:1) that had previously been mated with unexposed females to assure that they were fertile. All females from these matings were sacrificed 19 days after the first mating as determined by the presence of sperm in the vaginal smears. Upon sacrifice, the numbers of viable embryos, late deaths, early deaths (reabsorptions), and corpea lutea were determined. Preimplantation losses were also evaluated.

Statistical Methods

Student's t-test was employed when the variable mean of a control group was compared to that of an exposure group. When the probability that two means were from the same population was less than 0.05, they were considered significantly different.

When the mean values of a single parameter were compared among the exposure groups, one way analysis of variance (ANOVA) was employed. If a significant difference among the groups was indicated, the Bonferroni multiple comparison technique was used to compare individual groups. In these cases the probability limit of 0.05 was divided by the number of comparisons made to protect the confidence limit.

Differences among the groups based on histopathologic data (non-parametrically ranked) were examined by the Kruskall-Wallis test. A non-parametric multiple comparison technique proposed by Dun (30) was then used to compare the possible paired combinations of exposure groups.

Sister chromatid exchange data was subjected to square root transformation. This served to normalize the distribution of the data and resulted in greater homogeneity of variance. The Student's t test was then used to analyze the data. Bone marrow and peripheral blood lymphocyte population kinetics were analyzed using the Student's t-test after transformation of

the data from each animal to a mean cell cycle number. The mean cell cycle number = $\frac{1(I) + 2(II) + 3(III)}{100}$; where I, II, and III are the proportions of total cells scored (100) in the first, second, and third generations, respectively. The data on the percentage of abnormal sperm from each exposure group was analyzed by the Kruskall-Wallis non-parametric test and by one-way analysis of variance after arcsine transformation of the data.

The combined respiratory physiology, lung composition, and histopathologic endpoint assessment of individual animals resulted in 26 pulmonary variables from each animal which could be evaluated by correlation analysis. Linear associations were investigated between the three general classes of data (respiratory physiology, lung composition, and histopathology) within individual exposure groups.

The Pearson product-moment correlation was used when the parametric respiratory physiology and lung composition data were analyzed. Investigation of associations between the ranked pathology scores and the parametric data was conducted with the Spearman rank correlation test. However, the parametric respiratory physiology and lung composition data had to be transformed to a ranked system for this statistical analysis. The coefficients which resulted from these correlation procedures were transformed to t-statistics (29) and associations with probabilities of less than 0.05 were considered significant.

The data from the respiratory physiology studies and the lung composition analysis were also subjected to stepwise discriminant analysis. This stepwise procedure provided a reduced set of parameters, which most effectively discriminated the groups involved in a particular analysis. The first discriminant function was so derived as to maximize the ratio

This precluded complication of the data by excluding weights of animals which differed in age. One way analysis of variance indicated that the low and intermediate dose male animals were significantly ($p<0.0083$ by Bonferroni multiple comparison) heavier than the control males the first day of exposure. This difference was not noted again throughout the exposure regime. Male rats exposed to 4.0 ppm acrolein gained weight at a significantly reduced rate. Among the female exposure groups the weights of the 0.4 and 4.0 ppm animals differed significantly at the first weighing. At all subsequent weighings the weight of the high dose group was significantly less than that of the other groups among which there were no significant differences.

The weight changes of the individual groups during a six day period following removal of the animals from the chambers have been indicated in Figures 4 and 5. Groups of male and female rats removed from the 4.0 ppm chamber gained 35.1 (s.e. $\pm$ 4.34) and 24.0 (s.e. $\pm$ 1.58) gms, respectively, over the six day post-exposure period. Among the control, low, and intermediate exposure groups the six day post-exposure weight gain was less marked. The average weight gain among males and females in these groups was 12.3 and 7.6 gms, respectively. The weight of the rats from the high dose chamber differed significantly from those of the other exposure groups at the time of endpoint assessment. Also, the male rats from the 1.4 ppm chamber were significantly heavier than those from the control chamber six days post-exposure.

Organ Weight and Organ-to-Body Weight Ratios. The organ weight data provided in Tables 2 and 3 were derived from the animals designated for pathology from each exposure group. Additional lung data were available from those animals used in the respiratory physiology study, and these are

provided in Table 4.

Statistical analysis of the data in Table 2 indicated that the absolute organ weights of the low- and intermediate-dose rats did not differ significantly from those of the control group. Because the animals from the 4.0 ppm chamber were significantly lighter than those of the other groups (Table 2), most of the organ weights were also significantly less. However, the brain weights of these animals were not different from those of other groups. The lungs (with trachea attached) of the markedly smaller 4.0 ppm animals were significantly heavier than those from animals exposed to 0.4 and 1.4 ppm acrolein. When lung weights were examined as a function of body weight (Table 3), the ratio for the high dose group was markedly greater than that for any other group. Similar lung weights and lung-to-body weight ratios were also observed from animals in the respiratory physiology subgroups. The organ-to-body weight ratios of all organs, with the exception of the liver, in the high dose group were significantly greater than for animals from the other chambers.

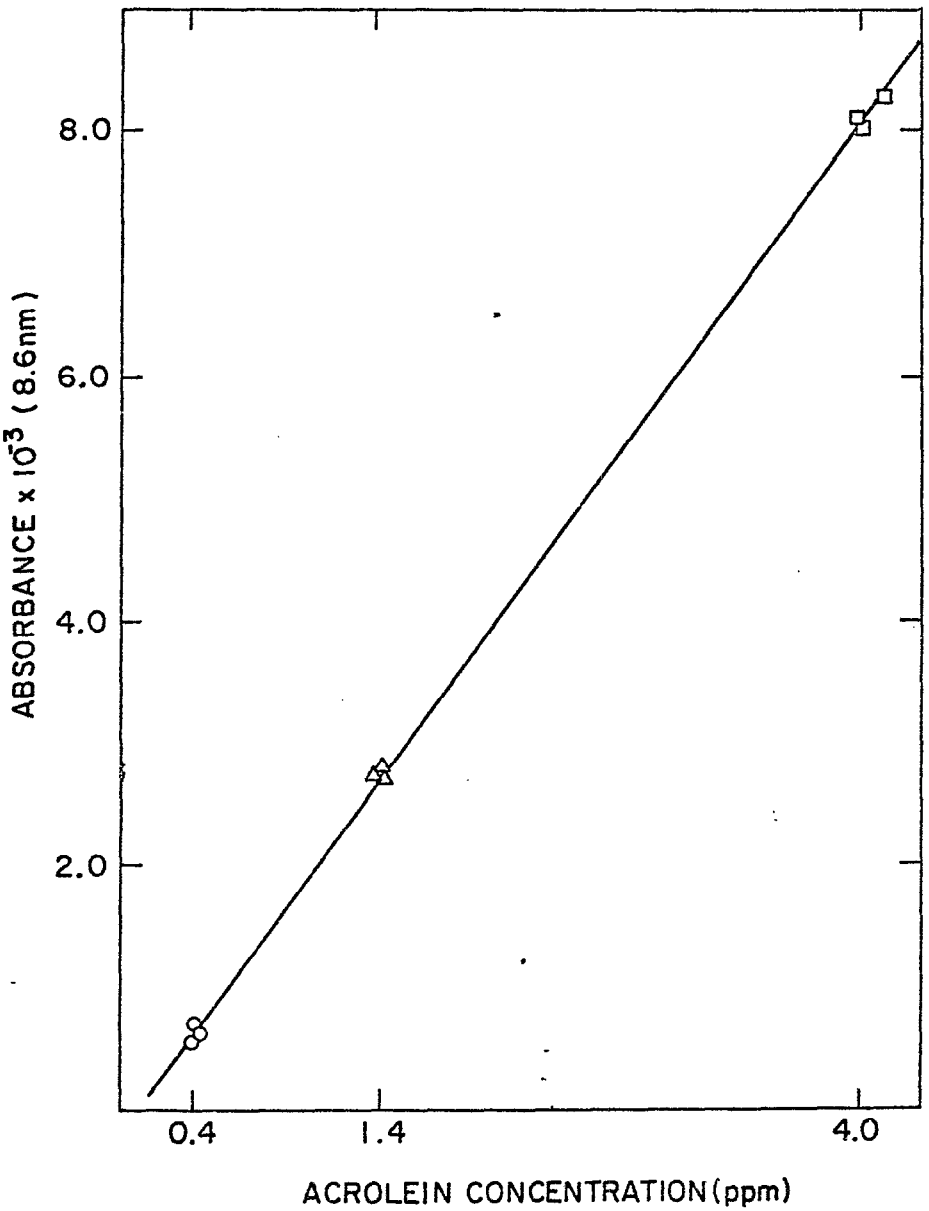


Figure 2: Linear regression plot of MIRAN 80-A absorbance values and concurrent acrolein concentrations determined by chemical analysis for each chamber (0.4 ppm (o), 1.4 ppm (Δ), and 4.0 ppm (□)) on three separate exposure days.

Table 1. Daily Mean Chamber Concentrations of Acrolein

Specified Concentration (ppm)	0.4	1.4	4.0
Exposure Day	Daily Mean Concentration (ppm)		
1	0.402	1.430	4.058
2	0.410	1.434	4.116
3	0.416	1.440	4.180
4	0.438	1.499	4.506
5	0.400	1.426	4.187
6	0.363	1.367	3.718
7	0.382	1.327	3.814
8	0.392	1.375	3.783
9	0.415	1.480	4.068
10	0.325	1.307	3.659
11	0.375	1.380	3.611
12	0.459	1.557	4.200
13	0.485	1.502	4.033
14	0.462	1.476	3.915
15	0.443	1.495	3.855
16	0.428	1.419	3.922
17	0.397	1.417	3.861
18	0.304	1.442	3.926
19	0.360	1.370	4.084
20	0.443	1.442	4.239
21	0.421	1.488	4.238
22	0.412	1.491	4.232
23	0.375	1.463	4.076
24	0.393	1.376	3.749
25	0.398	1.509	4.028
26	0.366	1.518	3.955
27	0.482	1.438	3.935
28	0.315	1.389	3.899
29	0.366	1.374	3.907
30	0.352	1.275	3.751
31	0.354	1.257	3.367
32	0.456	1.402	3.889
33	0.419	1.498	4.010
34	0.388	1.343	3.980

Table 1 - continued

	0.4	1.4	4.0
35	0.366	1.345	3.894
36	0.341	1.650	3.957
37	0.388	1.348	4.068
38	0.291	1.327	3.982
39	0.353	1.400	4.012
40	0.412	1.413	3.887
41	0.412	1.430	3.918
42	0.459	1.453	4.035
43	0.359	1.399	3.900
44	0.378	1.395	3.874
45	0.462	1.376	3.954
46	0.396	1.460	3.981
47	0.453	1.504	4.050
48	0.332	1.430	4.017
49	0.357	1.335	3.928
50	0.399	1.409	3.898
51	0.375	1.440	3.989
52	0.410	1.376	4.042
53	0.375	1.384	4.012
54	0.374	1.321	3.903
55	0.389	1.397	3.961
56	0.419	1.583	4.338
57	0.425	1.428	3.836
58	0.373	1.442	3.895
59	0.397	1.392	4.029
60	0.382	1.356	3.952
61	0.366	1.340	3.895
62	0.428	1.442	4.009
63	0.371	1.395	3.984
64	0.397	1.394	3.980
65	0.396	1.374	3.977
66	0.323	1.220	3.740
67	0.390	1.442	3.959
68	0.374	1.432	4.159
69	0.381	1.399	3.900
70	0.387	1.281	3.781

Table 1 - continued

	0.4	1.4	4.0
71	0.333	1.421	4.020
72	0.456	1.477	4.042
73	0.387	1.386	3.960
74	0.398	1.385	4.015
75	0.390	1.405	3.933
76	0.410	1.434	3.926
77	0.429	1.430	3.963
78	0.397	1.491	3.990
79	0.337	1.327	3.897
80	0.406	1.434	4.074
81	0.462	1.459	4.055
82	0.384	1.354	3.954
83	0.440	1.409	3.995
84	0.448	1.380	3.961
85	0.382	1.410	3.873
86	0.415	1.454	*
87	0.403	1.381	--
88	0.376	1.379	--

*High dose (4.0 ppm) exposures terminated

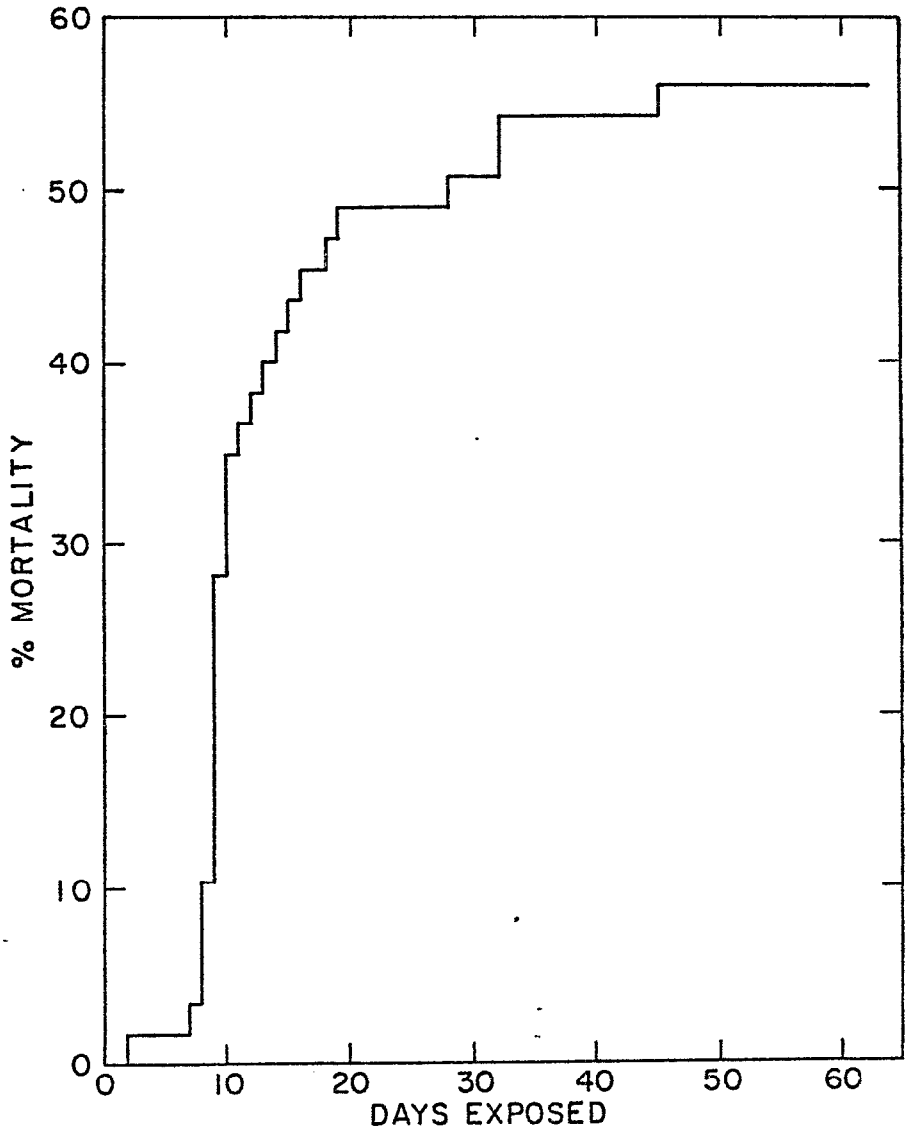


Figure 3: Mortality among male Fischer 344 rats exposed to 4.0 ppm acrolein for 62 days (6 hours/day, 5 days/week).

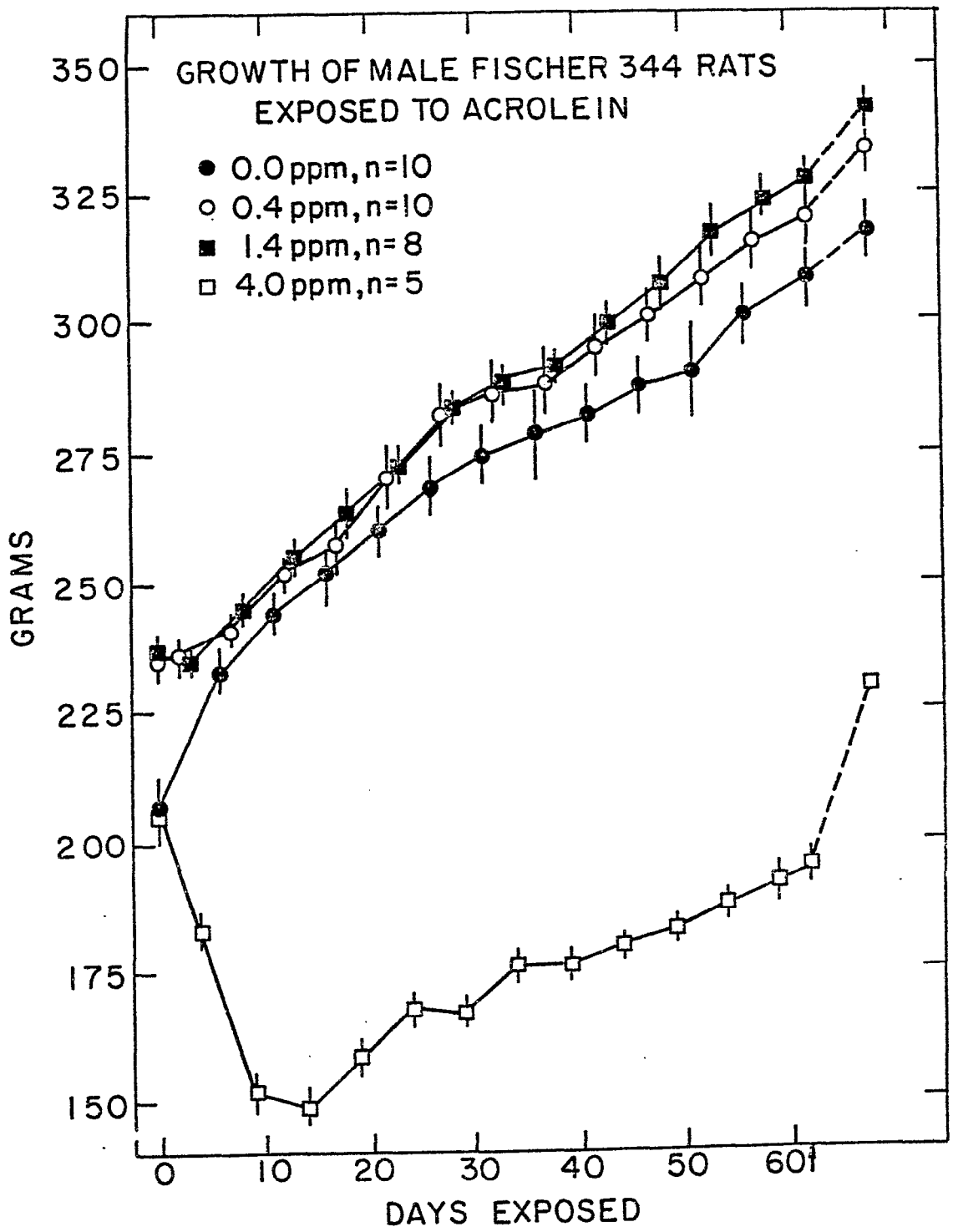


Figure 4: Weights of control and acrolein-exposed male Fischer 344 rats (↑; final exposure).

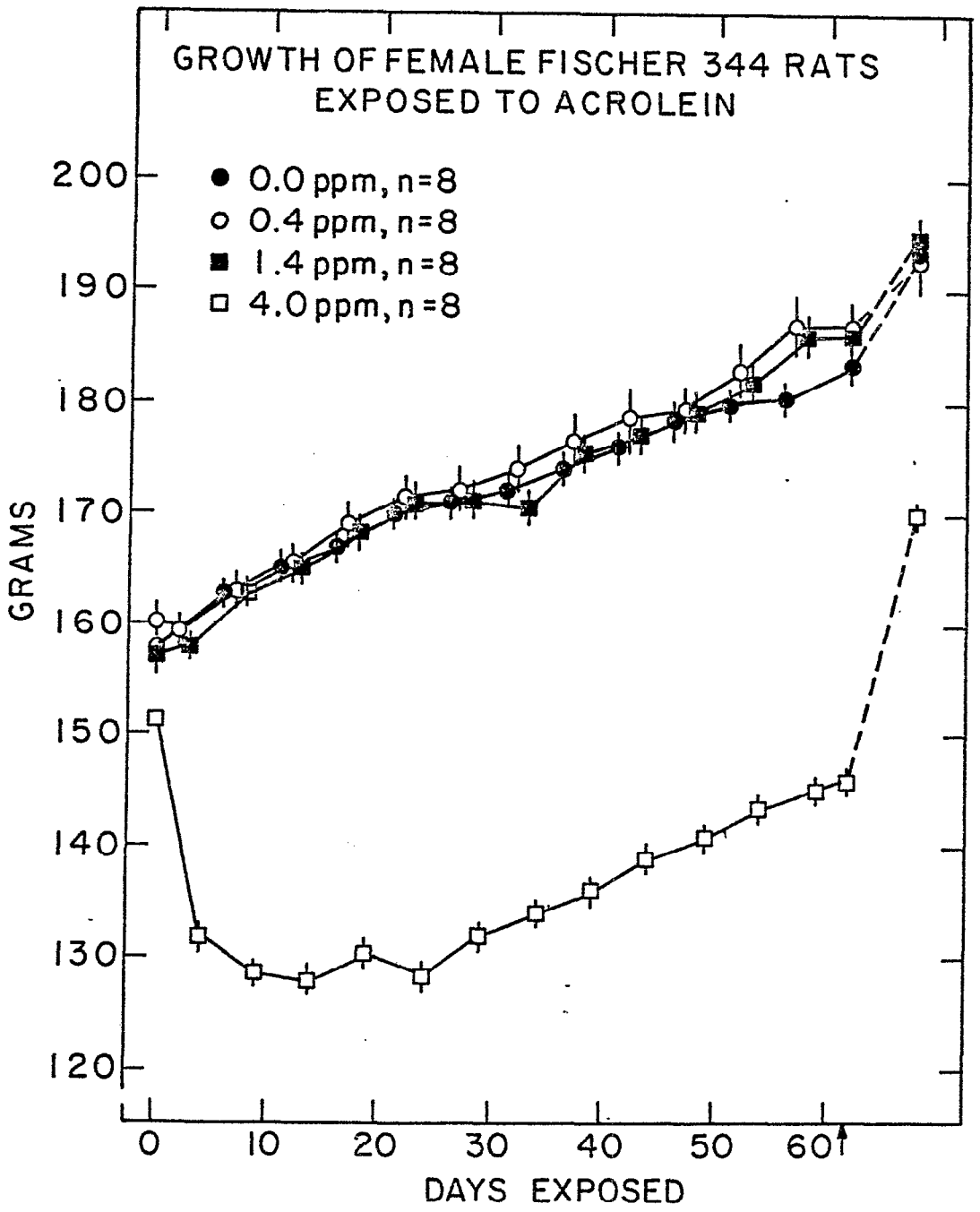


Figure 5: Weights of control and acrolein-exposed female Fischer 344 rats (↑; final exposure).

Table 2. Results and Statistical Analysis of Absolute Organ Weight and Body Weights of Fischer 344 Rats Exposed to Acrolein[a]

	ACROLEIN CONCENTRATION (ppm)			
	0.0	0.4	1.4	4.0
	n=8	n=8	n=8	n=5
LUNGS & TRACHEA	1.52(0.08)[b]	1.44(0.04)	1.34(0.06)	1.70(0.08)[c]
HEART	0.99(0.04)	1.04(0.04)	0.95(0.02)	0.82(0.04)[d]
SPLEEN	0.72(0.03)	0.68(0.04)	0.71(0.04)	0.48(0.02)[e]
KIDNEYS	2.42(0.08)	2.50(0.09)	2.30(0.06)	2.00(0.08)[d]
LIVER	11.44(0.46)	12.14(0.59)	11.12(0.21)	7.98(0.30)[e]
BRAIN	1.94(0.03)	1.95(0.05)	1.98(0.15)	1.76(0.02)
TESTIS	3.10(0.04)	2.94(0.12)	3.06(0.04)	2.62(0.12)[f]
BODY WEIGHT	340.4(8.2)	343.7(6.8)	327.4(5.1)	229.2(9.3)[e]

[a] Six hours/day, five days/week, 62 days.

[b] Grams, mean (±s.e.).

[c] Significantly different ($p<0.0083$) from 1.4 ppm group by Bonferroni multiple comparison technique.

[d] Significantly different ($p<0.0083$) from 0.0 and 0.4 ppm groups by Bonferroni multiple comparison technique.

[e] Significantly different ($p<0.0083$) from all other groups by Bonferroni multiple comparison technique.

[f] Significantly different ($p<0.0083$) from 0.0 and 1.4 ppm groups by Bonferroni multiple comparison technique.

Table 3. Results and Statistical Analysis of Organ-to-Body Weight Ratios of Fischer 344 Rats Exposed to Acrolein[a]

	ACROLEIN CONCENTRATION (ppm)			
	0.0	0.4	1.4	4.0
	n=8	n=8	n=8	n=5
LUNGS & TRACHEA	4.47(0.18)[b]	4.20(0.16)	4.09(0.18)	7.41(0.27)[c]
HEART	2.90(0.12)	3.02(0.08)	2.90(0.04)	3.59(0.20)[c]
SPLEEN	2.13(0.06)	1.96(0.12)	2.17(0.09)	2.10(0.09)
KIDNEYS	7.13(0.18)	7.27(0.20)	7.02(0.12)	8.72(0.14)[c]
LIVER	33.55(0.90)	35.29(1.50)	34.01(0.65)	34.78(0.74)
BRAIN	5.71(0.12)	5.67(0.11)	6.08(0.56)	7.70(0.28)[c]
TESTIS	9.13(0.16)	8.56(0.36)	9.36(0.10)	11.40(0.23)[c]

[a] Six hours/day, five days/week, 62 days.

[b] Organ weight (gms)/body weight (kgs), mean (±s.e.).

[c] Significantly different ($p<0.0083$) from all other exposure groups by Bonferroni multiple comparison technique.

Table 4. Results and Statistical Analysis of Lung Weight and Lung-to-Body Weight Ratios of Fischer 344 Rats,[a] Designated for Respiratory Physiology Studies After Exposure to Acrolein

	ACROLEIN CONCENTRATION			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=22	n=9
Lung Weight(gms)	1.29(0.02)[b]	1.28(0.03)	1.35(0.04)	1.71(0.06)[c]
Body Weight(gms)	326.1(2.7)	336.9(4.5)	330.5(3.6)	241.0(3.1)[c]
$\frac{\text{Lungs(gms)}}{\text{Body (kg)}}$	3.96(0.06)	3.81(0.07)	4.09(0.14)	7.10(0.28)[c]

[a] Six hours/day, five days/week, 62 days.

[b] Mean (±s.e.).

[c] Significantly different ($p<0.0083$) from all other groups using Bonferroni multiple comparison technique.

Respiratory Physiology

The Student's t-test was used to compare the pulmonary function data of each exposure group to that of the controls and the term "significant" for these analyses denotes a probability (p) of less than 0.05. During the function tests an occasional data point could not be measured, resulting in its omission from statistical analysis. The actual number of data points considered in each analysis has been provided in the respective tables. The pulmonary function data from all animals tested have been provided in Appendix E.

Parameters of Spontaneous Breathing. Several measurements of normal tidal breathing were taken on each animal prior to the forced ventilatory maneuvers (Table 5). The 4.0 ppm animals exhibited significant changes in V_T (+26%), f (-41%), and R_L (+83%) when compared to control animals. No statistically significant changes in ΔP_L and C_{DYN} were observed. The 0.4 and 1.4 ppm exposure concentrations did not significantly affect any of these physiological parameters. Normalization of C_{DYN} and R_L, to the FRC* of each animal, altered the respective dose-related patterns of response (Figure 6). While unadjusted C_{DYN} was unaffected at each exposure level, C_{DYN}/FRC decreased 7, 25, and 41% relative to the controls with each concentration increment. However, only the 4.0 ppm animals were significantly different from the control group. Normalization of R_L to FRC (Figure 6) eliminated the apparent R_L increase observed at 4.0 ppm. Dose related changes in R_L/FRC were not evident.

Heart Rate. No significant alterations in heart rate were observed at any acrolein concentration. Significant irregularities in the EKG

*Unless otherwise specified, FRC will refer to the dilution determined value.

patterns were observed only during ventricular systole (QRS interval: +20%) and total ventricular activity (QT interval: -11%) in the 4.0 ppm group (Table 6). No overt deformities, such as T wave inversions, were observed in the electrocardiographic displays (Figure 7).

Lung Volumes. Vital capacity, manometrically determined during the QSC maneuver, dilution derived TLC and FRC, and their arithmetically computed components, RV, IC, ERV, and IRV were compared in control and acrolein exposed animals.

Neon dilution was the primary technique used for the determination of lung volume (TLC_d) avoiding the confoundment of so-called "trapped" airspace. However, the concept of this non-communicating airspace was considered in the comparison of FRC_d (computed by TLC_d-IC) to FRC_b (computed from Boyle's law). The latter measurement includes the "trapped" volume in the estimate of FRC. No significant changes from the control "FRC_b-FRC_d" volumes were observed after exposure to 0.4 and 1.4 ppm acrolein (Figure 8). A 337% ($p \leq 0.05$) increase in this differential volume was seen in the 4.0 ppm group. No attempt was made to determine the contribution, if any, of abdominal gas to the estimate of "trapped" air volume.

Figure 9 illustrates the impact of each level of acrolein on the divisions of lung volume. A significant increase in RV (171%) and FRC (108%) occurred in the 4.0 ppm group, relative to control volumes. Similarly, significant changes were observed for TLC (+49%), VC (+27%), and IC (+29%) in the 4.0 ppm group. Figure 10 shows the RV, FRC, and VC after normalization to TLC_d. The TLC_d-normalized increases in RV and FRC were 91% ($p \leq 0.0001$) and 36% ($p \leq 0.005$) of control values, respectively. The normalized VC of the 4.0 ppm exposed rats was significantly less (13%, $p<0.0001$) than that of controls. The extremely large FRC of the 4.0 ppm

groups (+108%, Figure 9) was the primary contributor to the enlarged TLC and thus accounted for the relative decrease in VC/TLC.

"Parenchymal" Behavior and DLCO. Quasi-static compliance (QSC), reported as "steep" (QSC_{ss}) or "chord" (QSC_{cs}) slope, was increased 16% (ns) and 34% ($p<0.0003$), respectively in the high dose group (Table 7). If expressed as specific compliance (QSC/FRC) the degree of response at 4.0 ppm was -39% ($p<.01$) and -29% (ns), steep and chord slopes, respectively. Regardless of expression, no significant changes were observed in these parameters at 0.4 and 1.4 ppm. Inspection of the actual QSC curves revealed a slight, but not statistically significant shift of the 0.4 ppm curve to the right of the control curve (Figure 11). The curve for the intermediate exposure group was identical to the control curve. However, the 4.0 ppm group exhibited a curve significantly shifted upward and to the left of control accounting for the increased slopes of the unadjusted curves (Table 7). Curve slope differences were eliminated by normalization to VC (Figure 12). The apparent dose related increase in $DLCO_{sb}$ (diffusion capacity of the lung for CO) was reduced (e.g. +45%, $p<0.0001$) to -4% (ns) of control values in the 4.0 ppm group after normalization to TLC (Table 7).

Distribution of Ventilation. Moment analyses of the multibreath N_2 washout indicated that the distribution of ventilation was significantly altered in the 4.0 ppm exposure group (Table 8). The ratio of the first (M_1) and second (M_2) weighted moments of lung N_2 turnover to the overall lung N_2 turnover (M_0) are the moment ratios expressed in Table 8. The typical control washout pattern exhibited three phases, suggesting three general lung compartments with distinct emptying patterns (Figure 13). The low and intermediate exposure groups retained a typical pattern with no

apparent changes in compartmental emptying (Figure 13). However, the 4.0 ppm group appeared to have only two compartments, both emptying faster than any of the observed control rates (Figure 13).

Flow Volume Dynamics. The forced flow volume (MEFV) curves demonstrated significant acrolein related flow changes in the 0.4 and 4.0 ppm groups (Table 9; Figure 14). The flow volume curves for the 1.4 ppm animals were essentially identical to those of the controls. However, the direction of the flow changes for the low and high dose groups relative to control values were diametrically opposite. After exposure to 0.4 ppm the expiratory flow rates in the effort independent limb of the curve were significantly elevated. The PEF of the 0.4 ppm group was only slightly higher (+4.0%, ns) than that of the controls (Table 9). The flow rates for the 4.0 ppm group were significantly depressed at all lung volumes. The slight convex pattern (away from the volume axis) typical of the effort independent region of control MEFV curves was significantly increased in the low dose group ($\Delta EFR_{25}25$ = 184%, $p<0.001$), and was unchanged for the intermediate and high dose animals (Table 9).

Upstream airway resistance (R_{us}) was calculated by relating maximum expiratory airflows to static lung pressures (P_{st}) at 45% of lung volume. The P_L recorded during the slow QSC (V/P_L) maneuver was assumed to approximate P_{st}. Because the QSC technique required the imposition of a driving force, P_L was adjusted to 0 cm H_2O at RV (-10 cm H_2O) to produce an analog of a self-driven V/P curve. The R_{us} of the 0.4 ppm group was significantly reduced (15%) relative to that computed for the control group (Table 10). The 4.0 ppm group exhibited a 129% increase in R_{us} (Table 10); the 1.4 ppm group was unchanged from the controls. This apparent parabolic dose response relationship is consistent with the qualitative and

quantitative (flow) changes observed for the forced flow volume maneuver.

Table 5. Parameters of Spontaneous Breathing[a] of Fischer 344 Rats Exposed to Acrolein[b]

	Acrolein Concentration (ppm)			
	0.0	0.4	1.4	4.0
V_T(cc)				
n	24	23	22	9
Mean	1.80	1.84	1.79	2.27
s.e.	0.051	0.051	0.033	0.096
p value[c]	--	ns	ns	<<0.0001
ΔP_L(cm H_2O)				
n	23	23	22	9
Mean	8.97	8.37	8.20	10.97
s.e.	0.599	0.579	0.491	1.219
p value	--	ns	ns	ns
f(breaths/min)				
n	24	23	22	8
Mean	68	71	72	40
s.e.	3.3	3.2	4.4	4.6
p value	--	ns	ns	<<0.0001
V_E(cc/min)				
n	24	23	22	9
Mean	121	130	129	90
s.e.	5.9	7.4	9.6	10.1
p value	--	ns	ns	<0.02
R_L(cm H_2O/cc/sec)				
n	24	23	22	9
Mean	0.76	0.68	0.75	1.39
s.e.	0.073	0.072	0.092	0.218
p value	--	ns	ns	<0.003
C_{DYN}(cc/cm H_2O)				
n	24	23	22	9
Mean	0.24	0.24	0.20	0.26
s.e.	0.014	0.020	0.012	0.034
p value	--	ns	ns	ns

[a] Recorded over at least 10 tidal breaths

[b] Six hours/day, five days/week, 62 days

[c] Using Student's t test

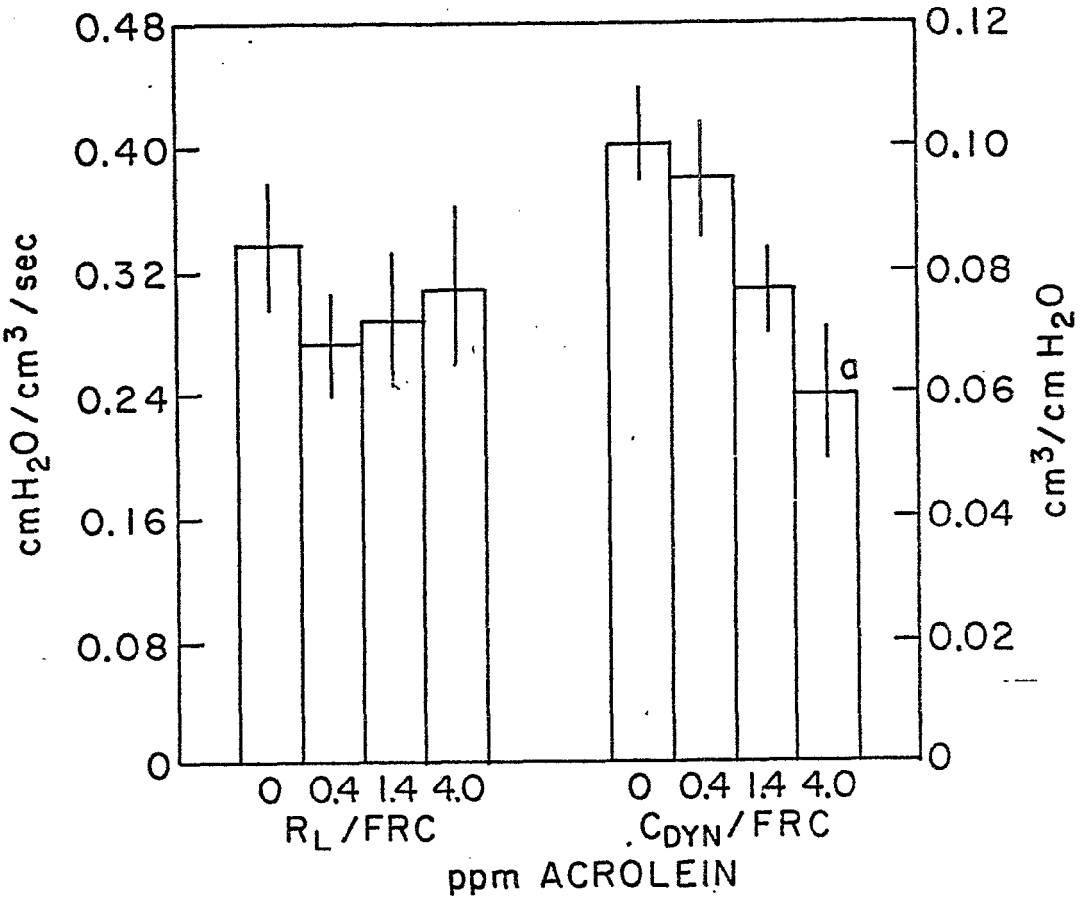


Figure 6: Pulmonary resistance (R_L) and dynamic compliance (C_{DYN}) normalized to the functional reserve capacity (FRC) of Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week). The number of rats in the 0.0, 0.4, 1.4, and 4.0 ppm exposure groups was 24, 24, 21, and 8, respectively.

[a]Significantly different from controls, $p<0.02$ using Student's t test.

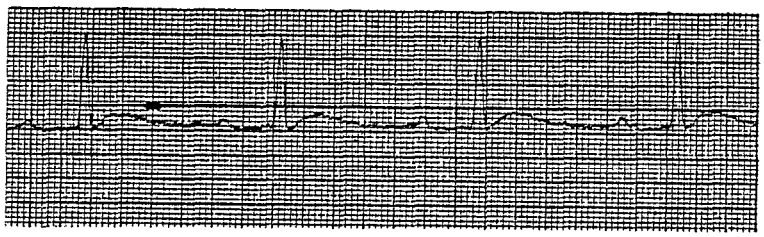
Table 6. Analysis of Electrocardiogram[a] Time Intervals of Fischer 344 Rats Exposed to Acrolein[b]

	Acrolein Concentration (ppm)			
	0.0	0.4	1.4	4.0
n	23	22	22	9
Heartbeats/Min.				
Mean	395	387	390	377
s.e.	11	10	12	12
p value[c]	--	ns	ns	ns
P-R (sec)				
Mean	0.0521	0.0548	0.0562	0.0551
s.e.	0.00111	0.00124	0.00080	0.00109
p value	--	ns	<.05	ns
QRS (sec)				
Mean	0.0175	0.0210	0.0185	0.0170
s.e.	0.00051	0.00075	0.00056	0.00071
p value	--	<.001	ns	ns
Q-T (sec)				
Mean	0.0680	0.0672	0.0647	0.0766
s.e.	0.00211	0.00195	0.00198	0.00288
p value	--	ns	ns	<.05

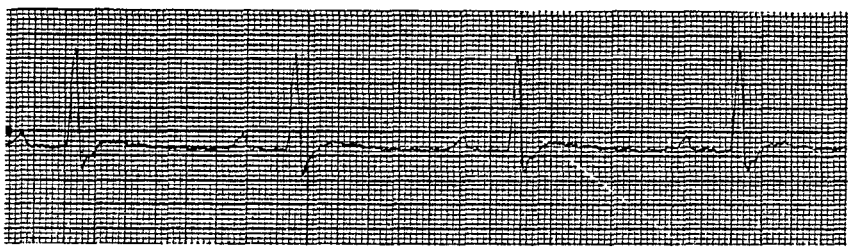
[a]Data taken from single series of cardiac impulses prior to pulmonary function tests.

[b]Six hours/day, five days/week, 62 days.

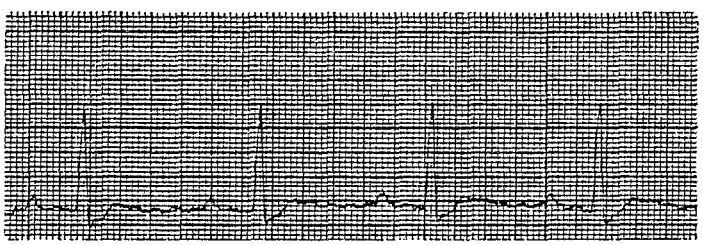
[c]Using Student's t-test.



CONTROL



0.4 ppm ACROLEIN



4.0 ppm ACROLEIN

Figure 7: Representative electrocardiograms of Fischer 344 rats exposed to 0.0, 0.4, and 4.0 ppm acrolein for 62 days (6 hours/day, 5 days/ week).

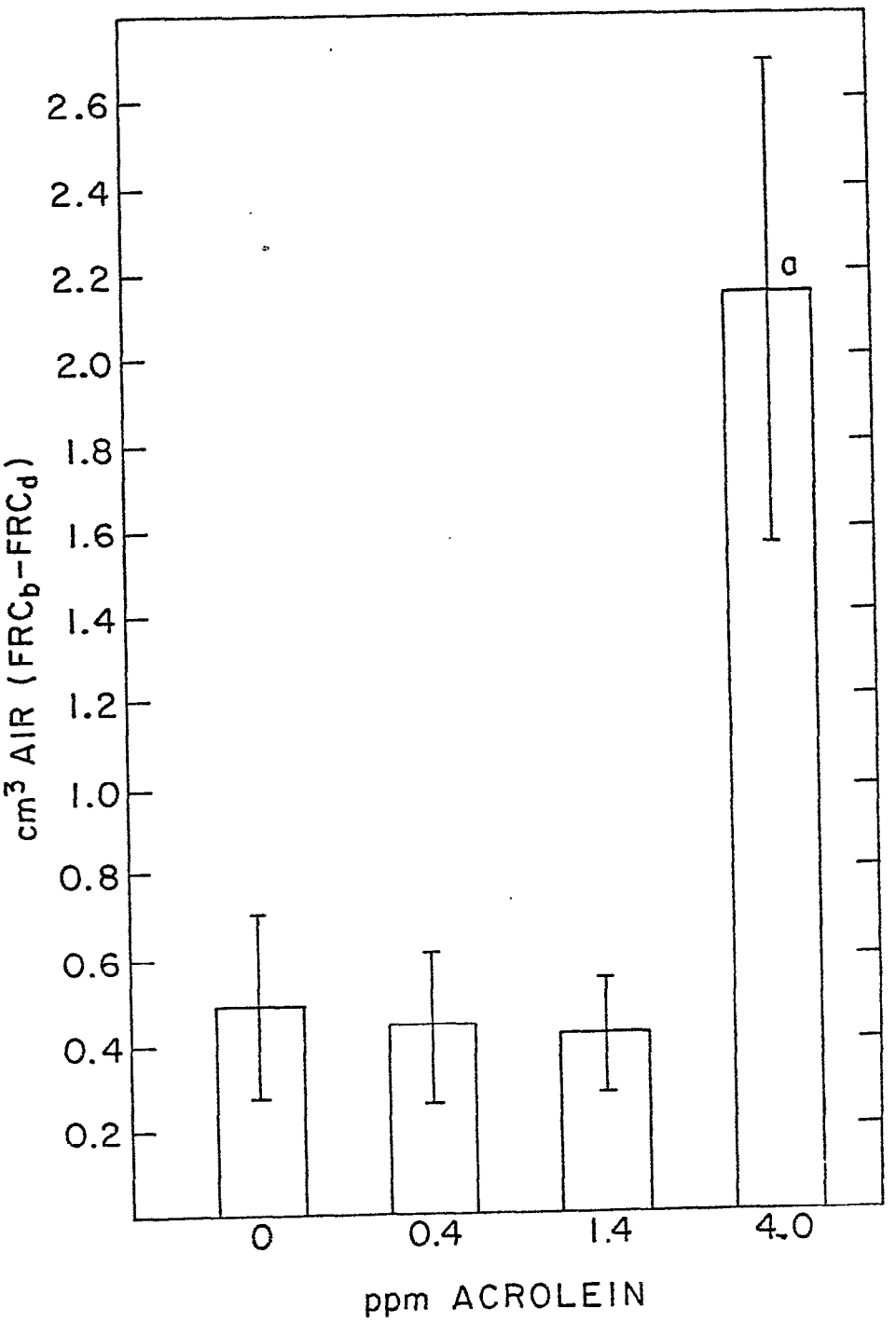


Figure 8: Trapped air in the lungs of Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week). Data presented are the means (+s.e.) of 24 control, 23 0.4 ppm, 21 1.4 ppm, and 8 4.0 ppm acrolein exposed rats.

FRC_b: Functional reserve capacity determined by Boyle's Law.

FRC_d: Functional reserve capacity determined by dilution.

[a]: Significantly different from controls, $p<0.001$ using Student's t-test.

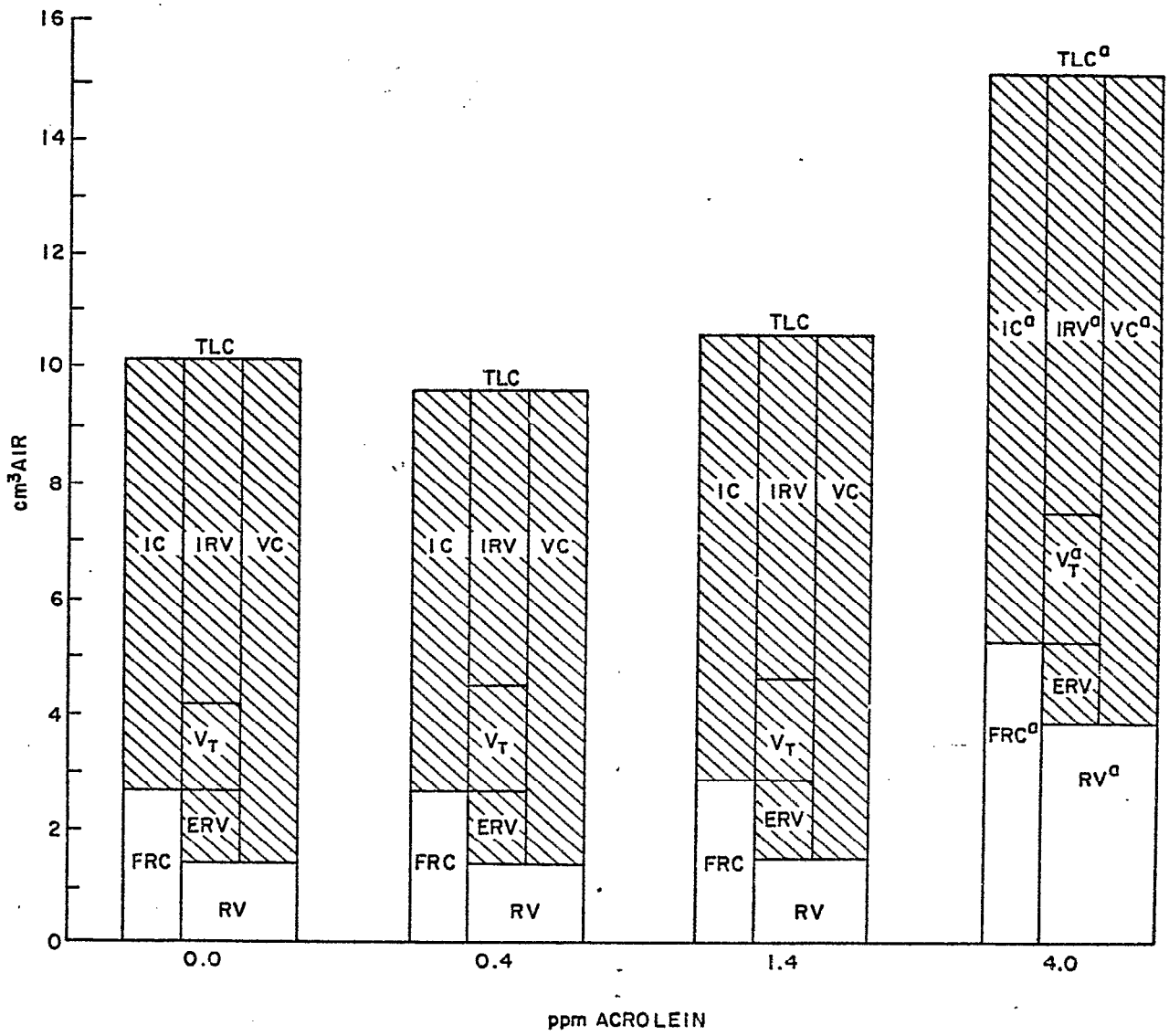


Figure 9: Divisions of lung volumes in Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week).

ERV: Expiratory reserve volume
FRC: Functional reserve capacity
IC: Inspiratory capacity
IRV: Inspiratory reserve volume
RV: Residual volume
VC: Vital capacity
V_T: Tidal volume
TLC: Total lung volume

^{a}: Significantly different from controls, $p<0.0001$, using Student's t test.

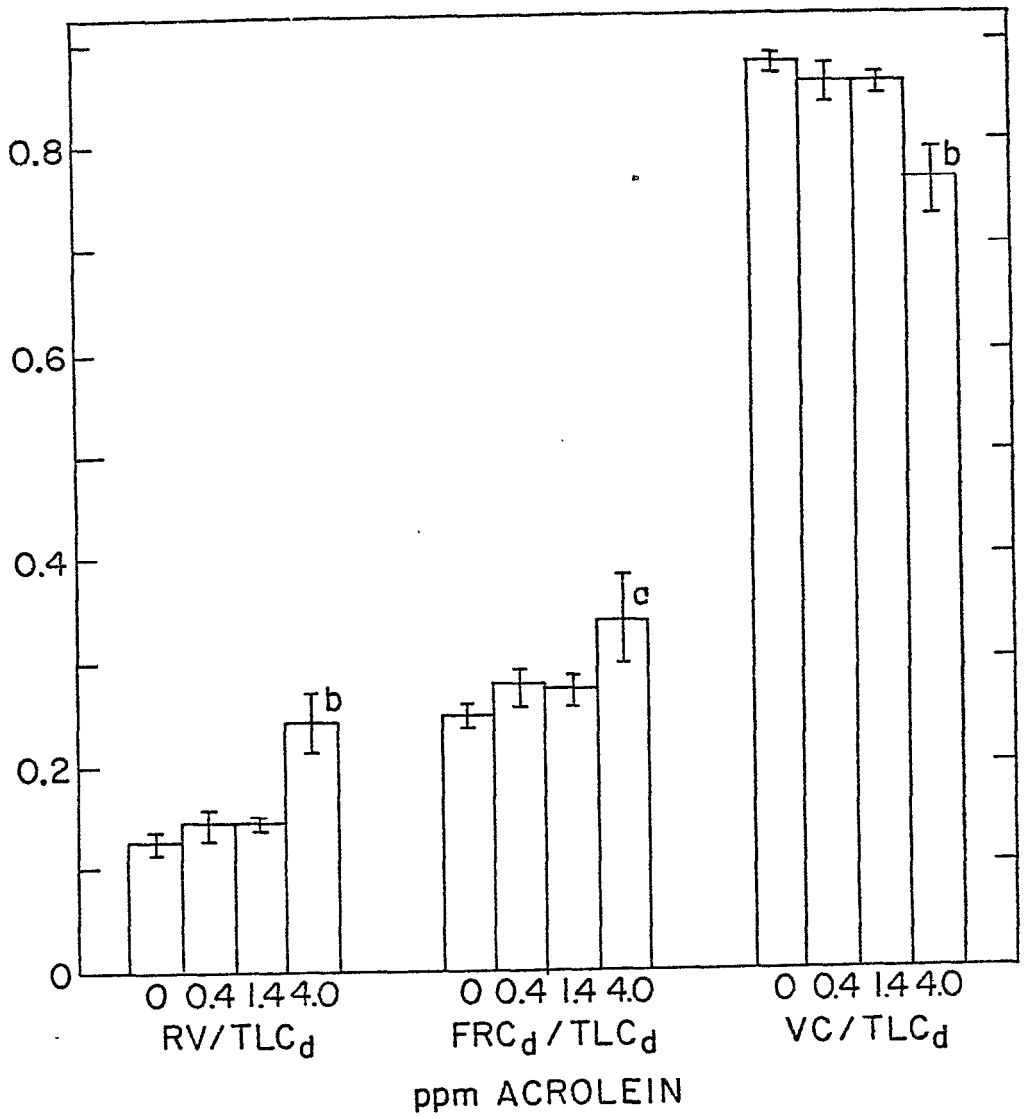


Figure 10: Normalized lung volumes of Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week). Data presented are the means (±s.e.) of 24, 24, 21, and 8, control, 0.4 ppm, 1.4 ppm, and 4.0 ppm acrolein exposed rats, respectively.

FRC: Functional reserve capacity
RV: Reserve volume
TLC: Total lung volume
VC: Vital capacity

a: Significantly different from controls, $p \leq 0.005$ using Student's t-test.
b: Significantly different from controls, $p \leq 0.0001$ using Student's t-test.

Table 7. Indices of Parenchymal Damage in Fischer 344 Rats Exposed to Acrolein[a]

	Acrolein Concentration (ppm)			
	0.0	0.4	1.4	4.0
QSC_{ss}				
n	23	23	22	9
Mean	0.83	0.73	0.77	0.96
s.e.	.049	.029	.048	.108
p value[b]	--	ns	ns	ns
QSC_{ss}/FRC[c]				
n	24	23	21	9
Mean	0.36	0.30	0.29	0.22
s.e.	.031	.029	.022	.035
p value	--	ns	ns	<0.01
QSC_{cs}				
n	23	23	22	9
Mean	0.56	0.56	0.58	0.75
s.e.	.027	.023	.027	.050
p value	--	ns	ns	<0.0003
QSC_{cs}/FRC[c]				
n	23	23	21	9
Mean	0.24	0.23	0.22	0.17
s.e.	.019	.021	.016	.025
p value	--	ns	ns	ns
$DLCO_{(sb)}$				
n	24	23	21	9
Mean	0.227	0.245	0.254	0.329
s.e.	.007	.006	.010	.026
p value	--	ns	ns	<<.0001
$DLCO_{(sb)}/TLC$[c]				
n	24	23	21	9
Mean	0.023	0.025	0.024	0.022
s.e.	.001	.000	.001	.001
p value	--	<.01	<.05	ns

[a]Six hours/day, five days/week, 62 days

[b]Using Student's t-test

[c]Dilution volumes

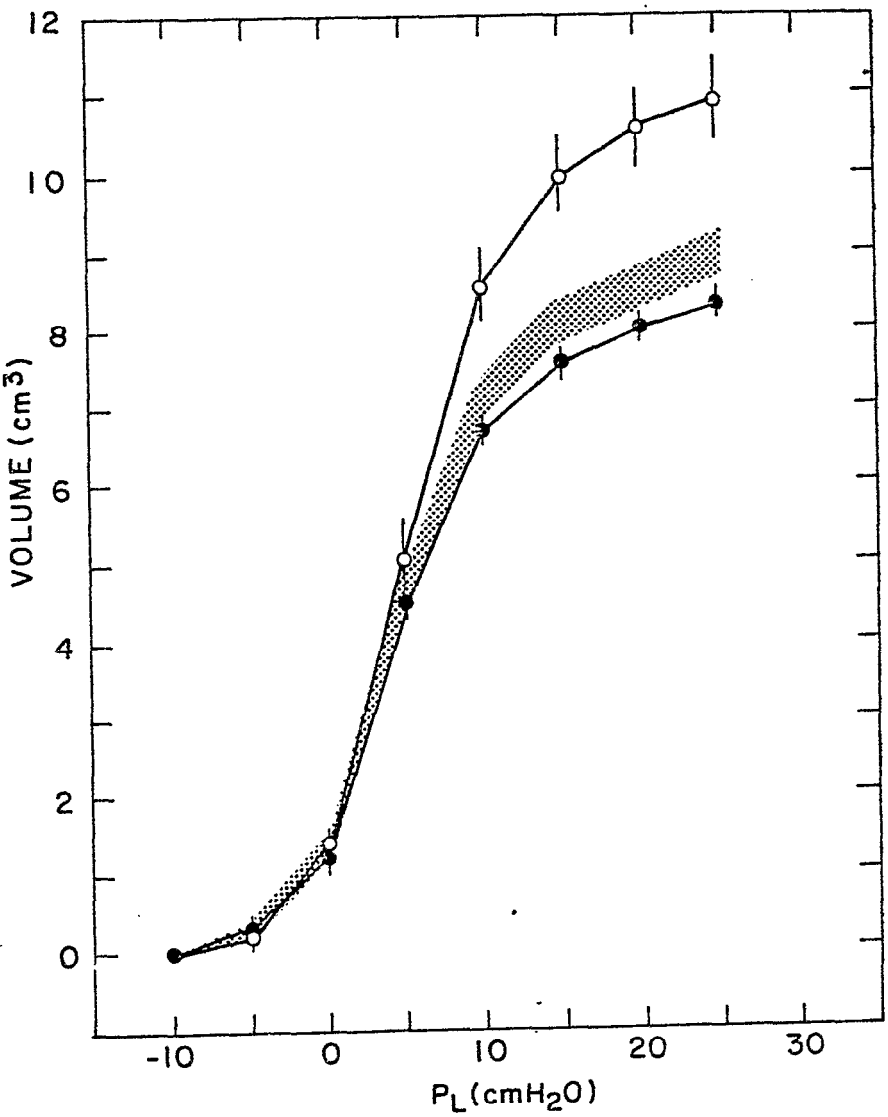


Figure 11: Quasi-static compliance of Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week). The means and s.e. bars of 24 controls, and 21 1.4 ppm exposed animals lie within the shaded area. The data from 23 rats exposed to 0.4 ppm acrolein (-●-) and 9 rats exposed to 4.0 ppm acrolein (-o-) are plotted separately.

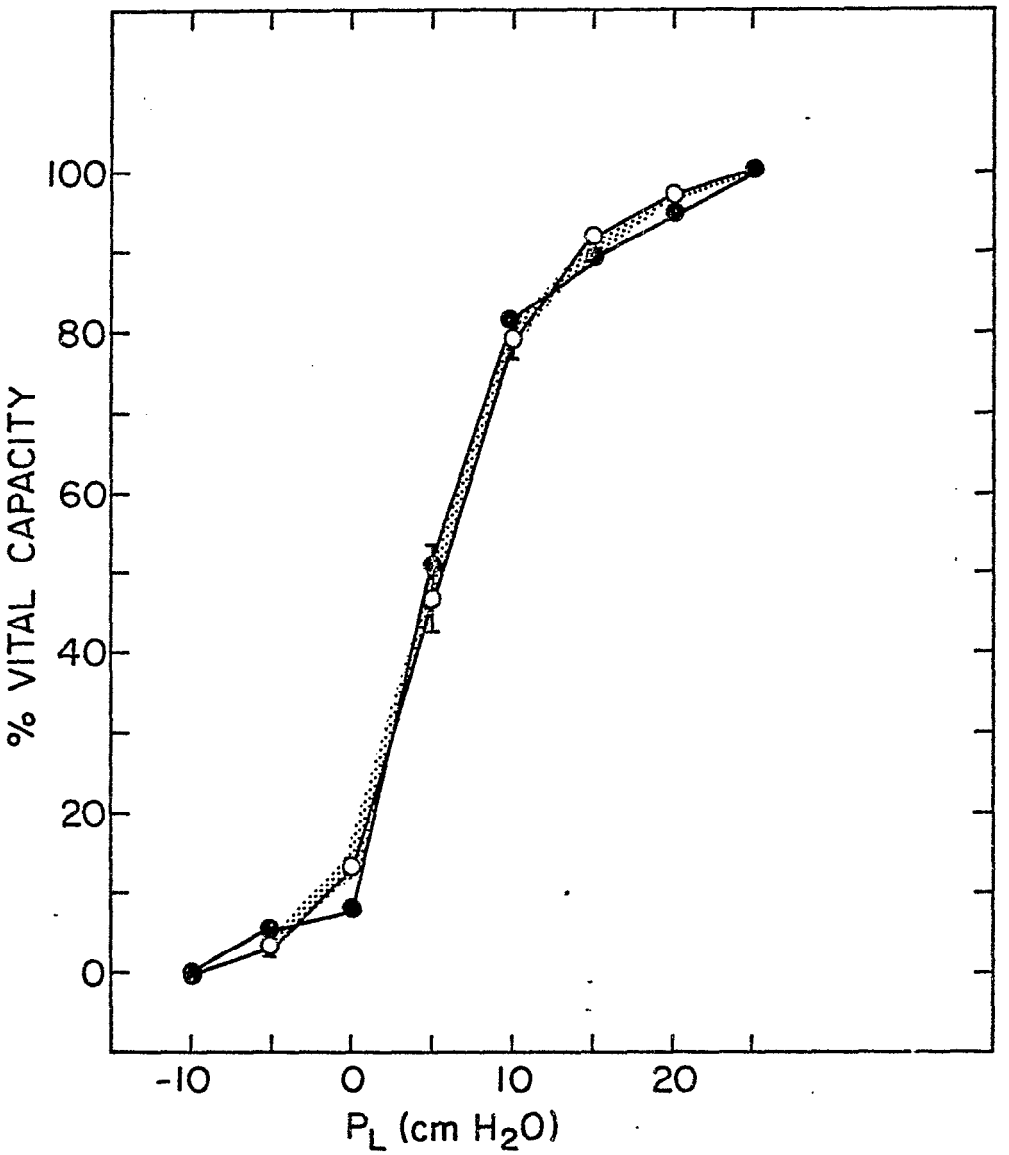


Figure 12: Quasi-static compliance as a function of vital capacity of Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week). The means and s.e. bars of 24 control and 23 1.4 ppm rats lie within the shaded area. The data from 23 rats exposed to 0.4 ppm acrolein (-●-) and 9 rats exposed to 4.0 ppm acrolein (-o-) are plotted separately.

Table 8. Moment Analysis of Multibreath N_2 Washout in Fischer 344 Rats Exposed to Acrolein[a]

	Acrolein Concentration (ppm)			
	0.0	0.4	1.4	4.0
	n=23	n=22	n=18	n=8
M_1/M_0				
Mean	12.43	12.64	12.13	10.18
s.e.	.368	.277	.390	.186
p value[b]	---	ns	ns	<.05
M_2/M_0				
Mean	290.34	298.83	277.01	214.28
s.e.	13.591	9.880	14.325	45.213
p value	---	ns	ns	<.05

[a] Six hours/day, five days/week, 62 days.

[b] Using Student's t-test.

M_0: Total area under the N_2 washout curve for 50 breaths.

$$M_0 = \sum_{j=1}^{50} X_j$$

$$M_1 = \sum_{j=1}^{50} j \cdot X_j$$

$$M_2 = \sum_{j=1}^{50} j^2 \cdot X_j$$

$j = 5$ (dilution no. + 1)

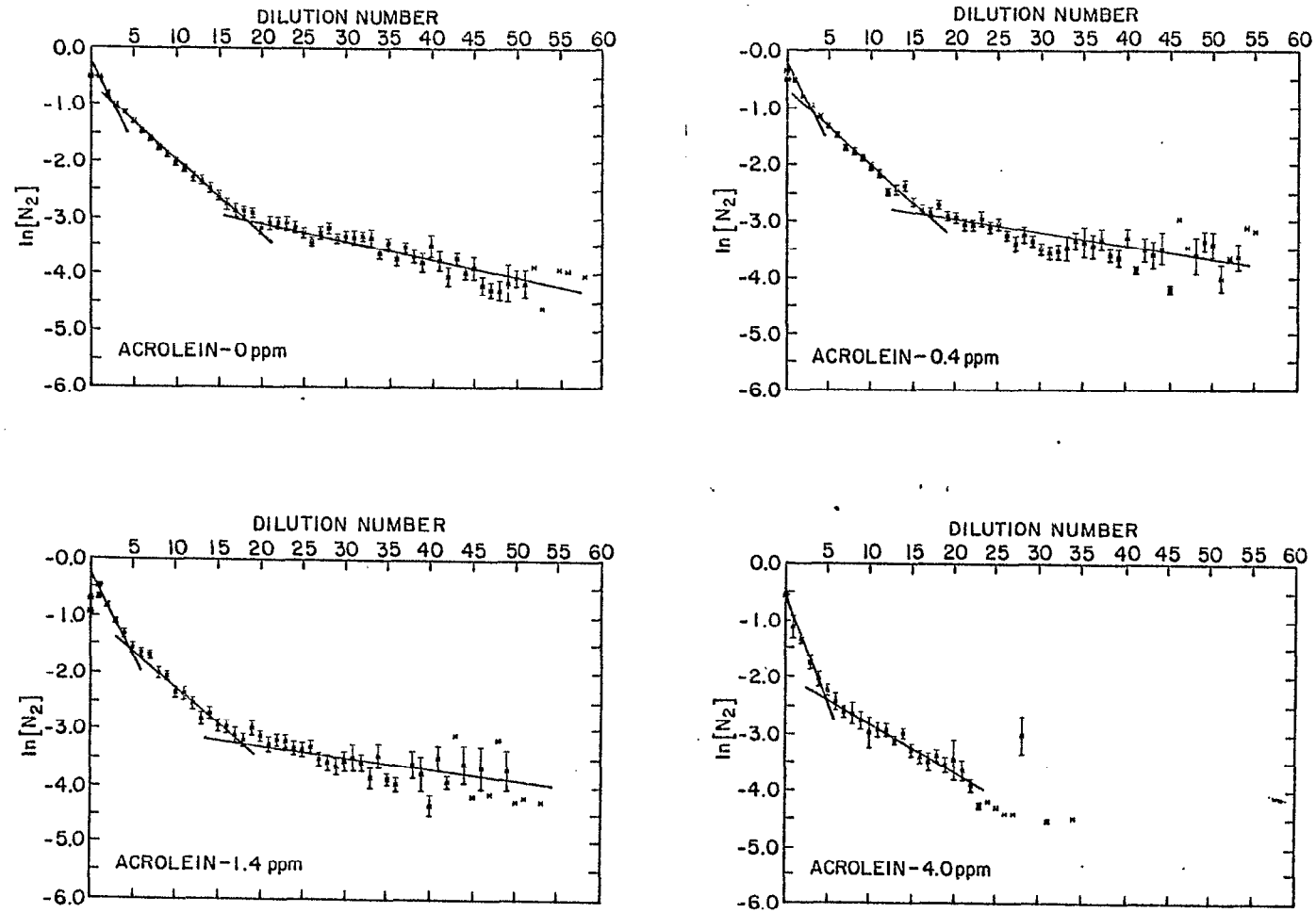


Figure 13: Multibreath N_2 washout curves for control and acrolein exposed Fischer 344 rats. Each graph is the mean (±s.e.) ln of the end-expiratory N_2 concentration versus the dilution number $\left[\frac{\text{breath \#} \cdot V_T}{FRC}\right]$. The slope of the lines approximates the time constant for N_2 turnover in each lung compartment.

Table 9. Statistical Analysis of Normalized Data Points on the MEFV Curve of Fischer 344 Rats Exposed to Acrolein[a]

	Acrolein Concentration (ppm)			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=22	n=9
PEF(VC/sec)				
Mean	11.42	12.65	11.25	7.63
s.e.	.327	.403	.313	.426
p value[b]	--	<.025	ns	<.001
EFR_{50}(VC/sec)				
Mean	9.9	11.6	10.2	5.8
s.e.	.36	.41	.36	.65
p value	--	<.0025	ns	<<.0001
EFR_{25}(VC/sec)				
Mean	5.7	7.8	6.0	2.8
s.e.	.36	.39	.44	.60
p value	--	.0002	ns	<.0001
EFR_{10}(VC/sec)				
Mean	2.7	3.9	3.0	0.89
s.e.	.28	.27	.34	.291
p value	--	.0030	ns	<.0001
ΔEFR_{25}(VC/sec)				
Mean	0.70	1.99	0.89	-0.17
s.e.	.244	.241	.313	.371
p value	--	<.0006	ns	ns

[a]Six hours/day, five days/week, 62 days.

[b]Using Student's t-test.

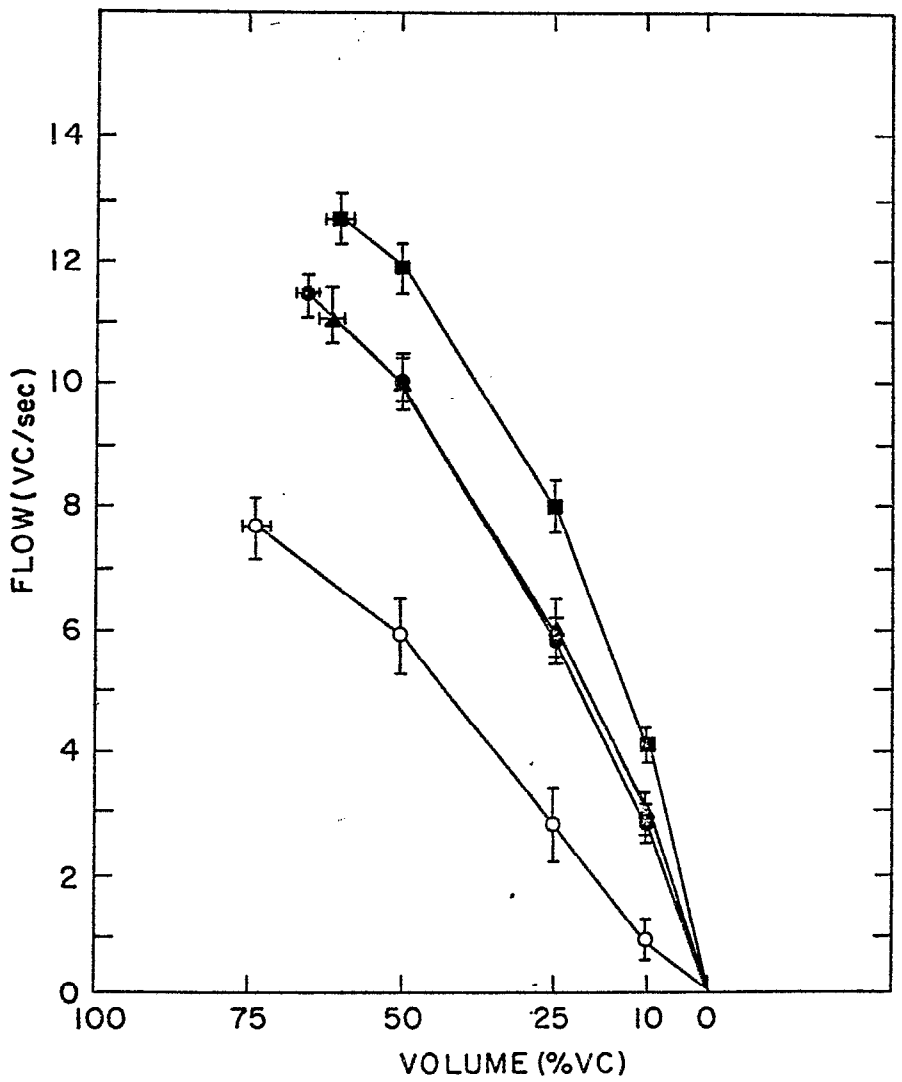


Figure 14: Maximum expiratory flow volume curves of Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week).

● control, n=24

■ 0.4 ppm acrolein, n=23

▲ 1.4 ppm acrolein, n=22

o 4.0 ppm acrolein, n=9

Table 10. Analysis of Upstream Airway Resistance[a] in Fischer 344 Rats Exposed to Acrolein[b]

	Acrolein Concentration (ppm)			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=21	n=9
% VC (FEV)				
Mean	54.8	56.1	55.8	55.7
s.e.	0.44	0.70	0.53	1.59
p value[c]	--	ns	ns	ns
V(cc/sec)				
Mean	80.7	90.8	84.1	46.8
s.e.	2.43	2.93	3.49	9.28
p value	--	≤.02	ns	<.001
P_LStat(cm H_2O)				
Mean	4.40	3.66	4.18	4.39
s.e.	0.218	0.236	.383	.534
p value	--	<.05	ns	ns
R_{us}(cm H_2O/cc/sec)				
Mean	0.182	0.154	0.174	0.416
s.e.	0.005	0.006	.008	.079
p value	--	<.005	ns	<.001

[a]Upstream airways are those distal or upstream of the theoretical equal pressure point achieved during forced expiration (MEFV).

[b]Six hours/day, five days/week, 62 days.

[c]Using Student's t-test.

Pathology Data

Selected tissues from three groups of animals were submitted to EPL for pathological examination. Group one consisted of animals found dead or moribund before completion of 62 exposure days. Lungs from all the animals in this group were sent to EPL which randomly selected specimens for examination. All animals in this group were from the 4.0 ppm acrolein chamber. The second group consisted of eight male animals from each exposure level designated as pathology animals. However, only five of the eight designated rats survived in the 4.0 ppm chamber. Group 3 was made up of the respiratory physiology animals. Selected tissues from all the animals used in the pulmonary assessment tests were submitted for pathological examination. These were studied to determine if possible correlations could be drawn between the pulmonary function and the pathology of individual animals. These specimens also provided an opportunity to determine whether or not the pulmonary function test regime resulted in any structural changes detectable at the light microscopy level.

Dead and Moribund Animals. Lungs of animals found dead or moribund displayed severe, acute bronchopneumonia; however, several areas of the lungs appeared unaffected. There was focal alveolar edema with sloughed cells in the bronchi and bronchioles. Most of the airways were actually plugged which would result in anoxia and death, even though there were healthy areas in the lungs. In addition to the pulmonary changes, there was tracheal edema with errosion of the mucosal epithelium.

Pathology Animals. Lungs of control rats displayed minimal to slight proliferations of lymphoid cells (Table 11) associated with a low grade chronic murine pneumonia. The presence of a slight acute or subacute alveolitis in some of these animals suggested a recent bacterial infection.

In a single animal there was an exacerbation of alveolitis to moderate acute bronchopneumonia and bronchitis. These changes were not severe in most cases and are mentioned for the purpose of baseline pulmonary pathology. Changes in organs other than the respiratory tract were negligible with the exception of focal mononuclear infiltrates in the livers of two rats (Table 11).

Rats exposed to 0.4 and 1.4 ppm acrolein had minimal to slight pulmonary lymphoid proliferations (Table 11) characteristic of chronic murine pneumonia. Rats from these exposure groups did not display pulmonary lesions attributable to acrolein exposure. Changes in the non-respiratory organs appeared incidental and were mild lesions commonly seen in laboratory rodents.

In the 4.0 ppm group, exposure related pulmonary lesions were seen in three out of five animals (Table 11); namely, bronchiolar epithelial necrosis and sloughing with a build-up of bronchiolar edema fluid and macrophages. Changes in the nonrespiratory organs including slight testicular atrophy in one rat and spleenic capsular fibrosis in another (Table 11) were considered incidental findings.

Respiratory Physiology Animals. Changes in the lungs of the control group were characteristic of a mild, low grade chronic pneumonia with focal acute or subacute alveolitis such as that described in the pathology animals. Again, these changes were not severe.

Lungs of animals in the 0.4 ppm respiratory physiology group, for the most part, resembled those of the controls. Three of the 23 rats in this group had slightly increased numbers of alveolar macrophages (Table 12), but this was not considered to be exposure related.

In the 1.4 ppm group, three animals (Table 12; 5703, 5747, and 5786) appeared to have an exposure-related pulmonary lesion which consisted of

bronchiolar epithelial necrosis and sloughed cells laying free in the lumen. Acrolein exposure resulted in increased numbers of alveolar macrophages and enhanced the degree of type II cell hyperplasia. Other changes associated with chronic murine pneumonia or a focal acute or subacute alveolitis appeared somewhat enhanced by acrolein exposure.

Lungs from the 4.0 ppm respiratory physiology group had lesions which appeared to be related to acrolein inhalation (Table 12). These included: 1) bronchiolar epithelial necrosis and sloughing, 2) bronchiolar edema with macrophages, and 3) focal edema. The numbers of alveolar macrophages also appeared to be somewhat increased. Edema in the trachea and peribronchial lymph nodes also appeared to be exposure related in this group as did acute rhinitis.

For the purpose of exploring possible correlations with the pulmonary function data and the lung composition data from individual animals, the severity of the pulmonary pathology in all of the respiratory physiology animals was scored. The values in Table 13 are the sums of the subjective values given to the various pulmonary lesions for each animal in Table 12. Also provided in Table 13 is the overall ordinal rank of each respiratory physiology animal. The frequency of each pathology score for the animals in each of the four exposure groups has been plotted in Figure 15. Although mild histological changes were observed in the control and 0.4 ppm animals, a dose related increase in pathological change was clearly evident at the higher exposure concentrations. Also, the broad range of intra-group variability in response to 1.4 and 4.0 ppm acrolein is apparent in Figure 15. Many of the animals in the 1.4 ppm group had scores which overlapped those of the controls, while three of the nine 4.0 ppm animals showed no histopathologic changes. The Kruskall-Wallis one way analysis of variance indicated a significant difference among the pathology scores of the four

exposure groups ($p<0.0039$). A multiple comparison procedure for non-parametric data (30) was employed to compare the individual groups. To maintain the appropriate confidence interval, the selected probability level of significance was divided by the number of comparisons (six). If $p<0.0083$ ($0.05\div6$) was selected as the level of significance, only the 0.4 group differed from the 1.4 and 4.0 ppm animals, reflecting the slightly lower than control scores in the 0.4 ppm group. However, if a $p<0.0167$ ($0.10\div6$) was assumed significant, the scores of the 1.4 and 4.0 ppm animals were statistically greater than those of both the control and the 0.4 groups.

TABLE 11: PATHOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

	Controls														Low Dose - 0.4 ppm													
ANIMAL NUMBER	5509	5510	5511	5512	5513	5514	5515	5585							5609	5610	5611	5612	5613	5614	5615	5616						
LUNG																												
Lymphoid Proliferations,																												
Perivascular and Peribronchiolar	2	1	1	1	2	1	2	2							1	2	1	3	1	2	1	1						
Alveolitis, Focal, Acute/Subacute	2	2	2	2	3		2	1							2	3	2	2	2	2	2	1						
Type II Cell Hyperplasia	1	1	1	1	1		1								1	2	1	1	1	1	1							
Granuloma, Focal			P																									
Bronchitis, Acute					2																							
Bronchopneumonia, Acute					3																							
Edema, Focal																												
Pleuritis, Focal																												
Bronchiolar Epithelial Necrosis																												
and Sloughing																												
Bronchiolar Edema and Macrophages																												
TRACHEA	X		X	X	X	X	X	X							X	X	X	X	X	X		X						
Chronic Tracheitis		1																			1							

EPL | Experimental Pathology Laboratories, Inc.

Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

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Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

	Controls														Low Dose - 0.4 ppm													
ANIMAL NUMBER	5509	5510	5511	5512	5513	5514	5515	5585							5609	5610	5611	5612	5613	5614	5615	5616						
PERIBRONCHIAL LYMPH NODE		N				X									N	N		N		X	X							
Necrosis, Focal	2				2																							
Lymphoid Hyperplasia	2			1	3		2	2									2		1			1						
Reticuloendothelial Hyperplasia			1																									
Congestion																			1			1						
NASAL TURBINATE	X	X	X	X	X	X														X	X	X						
Submucosal Lymphoid Aggregates							2	1							2	2	2	1	1									
BRAIN	X	X	X	X	X	X	X	X							X	X	X	X	X	X	X	X						
KIDNEY	X	X	X	X	X	X	X	X							X	X	X	X	X	X	X	X						
LIVER		X		X	X	X	X	X							X	X	X	X	X	X	X	X						
Mononuclear Cell Infiltrate, Focal	1		1																									
SPLEEN	X	X	X	X	X	X	X	X							X	X	X	X	X	X	X	X						
Capsular Fibrosis, Focal																												

EPL | Experimental Pathology Laboratories, Inc.

Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

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Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

	Controls														Low Dose - 0.4 ppm													
ANIMAL NUMBER	5509	5510	5511	5512	5513	5514	5515	5585							5609	5610	5611	5612	5613	5614	5615	5616						
TESTIS	X	X	X	X	X	X	X	X							X	X	X	X	X	X	X	X						
Atrophy																												
HEART	X	X	X	X	X	X	X	X							X	X	X	X	X	X	X	X						
Chronic Myocarditis, Focal																												

EPL
Experimental Pathology Laboratories, Inc.

Key: P = Present
1 = Minimal
5 = Severe/High
N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

TABLE 11: PATHOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

	Intermediate Dose - 1.4 ppm													High Dose - 4.0 ppm												
ANIMAL NUMBER	5709	5710	5711	5712	5713	5714	5715	5716						5809	5810	5812	5813	5815								
LUNG																										
Lymphoid Proliferations,																										
Perivascular and Peribronchiolar	1	2	1	1	2	2	1	1						2	1	1	2	1								
Alveolitis, Focal, Acute/Subacute					2	3									2			1								
Type II Cell Hyperplasia					2	1																				
Granuloma, Focal																										
Bronchitis, Acute																										
Bronchopneumonia, Acute																										
Edema, Focal															2											
Pleuritis, Focal															2											
Bronchiolar Epithelial Necrosis																										
and Sloughing															1	3										
Bronchiolar Edema and Macrophages															2	3		1								
TRACHEA	X	X	X	X	X	X	X	X						X	X	X	X	X								
Chronic Tracheitis																										

EPL | Experimental Pathology Laboratories, Inc.

Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

TABLE 11: PATHOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

	Intermediate Dose - 1.4 ppm														High Dose - 4.0 ppm												
ANIMAL NUMBER	5709	5710	5711	5712	5713	5714	5715	5716							5809	5810	5812	5813	5815								
PERIBRONCHIAL LYMPH NODE	X	N	X	N			N								N		N		X								
Necrosis, Focal																											
Lymphoid Hyperplasia					4	1		1								3		1									
Reticuloendothelial Hyperplasia																											
Congestion																1											
NASAL TURBINATE	X	N		X				X								X			X								
Submucosal Lymphoid Aggregates			2		3	1	1								2		3	2									
BRAIN	X	X	X	X	X	X	X	X							X	X	X	X	X								
KIDNEY	X	X	X	X	X	X	X	X							X	X	X	X	X								
LIVER		X	X	X	X	X		X								X	X	X	X								
Mononuclear Cell Infiltrate, Focal	1						1								1												
SPLEEN		X	X	X	X	X	X	X							X	X	X	X	X								
Capsular Fibrosis, Focal	2																										

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Key: P = Present, 1 = Minimal, 5 = Severe/High, N = No Section, 2 = Slight, I = Incomplete Section, A = Autolysis, 3 = Moderate, X = Not Remarkable, 4 = Moderately Severe/High

TABLE 11: PATHOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

	Intermediate Dose - 1.4 ppm														High Dose - 4.0 ppm												
ANIMAL NUMBER	5709	5710	5711	5712	5713	5714	5715	5716							5809	5810	5812	5813	5815								
TESTIS	X	X	X	X	X	X	X								X	X	X	X	X								
Atrophy								2																			
HEART	X	X	X	X	X	X	X								X	X	X	X	X								
Chronic Myocarditis, Focal								2																			

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Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

Controls

ANIMAL NUMBER	5501	5502	5503	5517	5518	5519	5542	5543	5544	5546	5548	5549	5550	5551	5552	5553	5554	5555	5556	5558	5559	5588	5591	5598				
LUNG										N	N	N										N						
Lymphoid Proliferations,																												
Perivascular and Peribronchiolar	1	2	1	2	2	2	2	2	2				1	1	1	1	1	1	1	2	1		1	2				
Alveolitis, Focal, Acute/Subacute	2	2	1		2	2	2							1										1				
Type II Cell Hyperplasia		1	1		1	1	1																					
Alveolar Macrophages																												
Bronchiolar Epithelial Necrosis																												
and Sloughing																												
Granuloma, Focal																												
Hemmorhage, Focal																												
Bronchiolar Edema and Macrophages																												
Edema, Focal																												
Chronic Pleuritis																												
TRACHEA	X		X	X	X	X	X	X	X	X	X	X	N	X	X	X	X		X	X	X	X	X	X				
Chronic Tracheitis		1																1										
Edema																												

EPL | Experimental Pathology Laboratories, Inc.

Key: P = Present, 1 = Minimal, 5 = Severe/High, N = No Section, 2 = Slight, I = Incomplete Section, A = Autolysis, 3 = Moderate, X = Not Remarkable, 4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

Controls

ANIMAL NUMBER	5501	5502	5503	5517	5518	5519	5542	5543	5544	5546	5548	5549	5550	5551	5552	5553	5554	5555	5556	5558	5559	5588	5591	5598
PERIBRONCHIAL LYMPH NODE					N				N	X	X			X	X	N	X	X	X	X			X	
Lymphoid Hyperplasia	3			3			2	2				1	2								1			1
Congestion		2	1			1																1		
Reticuloendothelial Hyperplasia																								
Edema																								
NASAL TURBINATE		N	N	N	N	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	X	X
Submucosal Lymphoid Aggregates	1																		1		1			
Acute Rhinitis																								

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Experimental Pathology Laboratories, Inc.

Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

Low Dose - 0.4 ppm

ANIMAL NUMBER	5601	5602	5617	5618	5619	5642	5643	5644	5645	5646	5649	5650	5652	5655	5656	5657	5687	5692	5693	5694	5695	5696	5697					
LUNG																												
Lymphoid Proliferations,																												
Perivascular and Peribronchiolar	2	1	2	1	1	2	2	2	1	2	2	2	1	1	1	1	1	2	2	1	1	1	2					
Alveolitis, Focal, Acute/Subacute	3					1	1			1		1											2					
Type II Cell Hyperplasia	2					1	1					1											1					
Alveolar Macrophages						1					1								1									
Bronchiolar Epithelial Necrosis																												
and Sloughing																												
Granuloma, Focal																												
Hemmorhage, Focal																												
Bronchiolar Edema and Macrophages																												
Edema, Focal																							.					
Chronic Pleuritis																												
TRACHEA	X	X	X	X	X	X	X	X	X	X	X	X	X	N	X	X	X	X	X	X	X	X	X					
Chronic Tracheitis																					.							
Edema																												

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Key: P = Present
. 1 = Minimal
5 = Severe/High
N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

Low Dose - 0.4 ppm

ANIMAL NUMBER	5601	5602	5617	5618	5619	5642	5643	5644	5645	5646	5649	5650	5652	5655	5656	5657	5687	5692	5693	5694	5695	5696	5697
PERIBRONCHIAL LYMPH NODE				N	X	N		N	X	X		N	N	N			N		N	N	N		N
Lymphoid Hyperplasia	1														2			2				1	
Congestion		2	2				1				1					1							
Reticuloendothelial Hyperplasia																							
Edema																							
NASAL TURBINATE	X	N	N	N	X	X	X	X	X	X	X	X	X			X	N	X	X	X	N	X	
Submucosal Lymphoid Aggregates														1	1								2
Acute Rhinitis																							

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Key: P = Present, 1 = Minimal, 5 = Severe/High, N = No Section, 2 = Slight, I = Incomplete Section, A = Autolysis, 3 = Moderate, X = Not Remarkable, 4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

Intermediate Dose - 1.4 ppm

ANIMAL NUMBER	5701	5702	5703	5742	5743	5744	5745	5746	5747	5749	5751	5752	5753	5754	5755	5756	5757	5758	5785	5786	5793	5798	5799					
LUNG																												
Lymphoid Proliferations,																												
Perivascular and Peribronchiolar	2	2	1	2	2	1	3	2	2	2	2	1	1	2	1	2	1	2	1	2	2	1	2					
Alveolitis, Focal, Acute/Subacute	2	3		2	2		3	2	2	1									1	1	2		2					
Type II Cell Hyperplasia	2	3		1	1		3	1	1											1	1		2					
Alveolar Macrophages	2	2		2	2		2		1		1				1			1			1		1					
Bronchiolar Epithelial Necrosis																												
and Sloughing			2						1											1								
Granuloma, Focal					P																							
Hemmorhage, Focal														2														
Bronchiolar Edema and Macrophages																												
Edema, Focal																												
Chronic Pleuritis																												
TRACHEA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X					
Chronic Tracheitis																		1										
Edema																												

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Key: P = Present
1 = Minimal
5 = Severe/High
N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

Intermediate Dose - 1.4 ppm

ANIMAL NUMBER	5701	5702	5703	5742	5743	5744	5745	5746	5747	5749	5751	5752	5753	5754	5755	5756	5757	5758	5785	5786	5793	5798	5799
PERIBRONCHIAL LYMPH NODE		X		N	X		N		N				N	N	X	X						N	N
Lymphoid Hyperplasia	3		2									2						2					
Congestion						2		2			2						1	2	2	1	1		
Reticuloendothelial Hyperplasia			3							1													
Edema																							
NASAL TURBINATE	N	N	N	X	X		X		X	X	X		N	X	X		X	X	X	X	X	N	X
Submucosal Lymphoid Aggregates						2		1				2				1	*						
Acute Rhinitis																							

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Key: P = Present; 1 = Minimal; 5 = Severe/High; N = No Section; 2 = Slight; I = Incomplete Section; A = Autolysis; 3 = Moderate; X = Not Remarkable; 4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

High Dose - 4.0 ppm

ANIMAL NUMBER	5801	5818	5842	5843	5845	5859	5886	5889	5890
LUNG									
Lymphoid Proliferations,									
Perivascular and Peribronchiolar	2	1	2	2	2	2	1	2	2
Alveolitis, Focal, Acute/Subacute								3	3
Type II Cell Hyperplasia								2	3
Alveolar Macrophages	3			1			2	2	3
Bronchiolar Epithelial Necrosis									
and Sloughing	2			2				2	2
Granuloma, Focal									
Hemmorhage, Focal									
Bronchiolar Edema and Macrophages	3			3			2	3	1
Edema, Focal	3			1		2			
Chronic Pleuritis								3	
TRACHEA	X	X	X	X					X
Chronic Tracheitis									
Edema					2	2	2	2	

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Experimental Pathology Laboratories, Inc.

Key: P = Present
1 = Minimal
5 = Severe/High
N = No Section
2 = Slight
I = Incomplete Section
A = Autolysis
3 = Moderate
X = Not Remarkable
4 = Moderately Severe/High

TABLE 12: PHYSIOLOGY ANIMALS

HISTOPATHOLOGY INCIDENCE TABLE

Brookhaven National Laboratory
National Toxicology Program
Acrolein Study
Male Rats

High Dose - 4.0 ppm

ANIMAL NUMBER	5801	5818	5842	5843	5845	5859	5886	5889	5890
PERIBRONCHIAL LYMPH NODE	N								
Lymphoid Hyperplasia			1		2		2		
Congestion		2						2	1
Reticuloendothelial Hyperplasia									
Edema				2		2			
NASAL TURBINATE		X	X	N		X			X
Submucosal Lymphoid Aggregates					2				
Acute Rhinitis	3						2	3	

EPL
Experimental Pathology Laboratories, Inc.

Key: P = Present, N = No Section, A = Autolysis, X = Not Remarkable
1 = Minimal, 2 = Slight, 3 = Moderate, 4 = Moderately Severe/High
5 = Severe/High, I = Incomplete Section

Table 13. Pulmonary Pathology Scores and Overall Ordinal Rank of all Fischer 344 Rats which Completed Respiratory Physiology Function Tests after Exposure to Acrolein[a]

0.0 ppm ACROLEIN			0.4 ppm ACROLEIN			1.4 ppm ACROLEIN			4.0 ppm ACROLEIN		
Animal Number	Pulmonary Pathology Score	Rank	Animal Number	Pulmonary Pathology Score	Rank	Animal Number	Pulmonary Pathology Score	Rank	Animal Number	Pulmonary Pathology Score	Rank
5501	2	50.0	5601	5	66.0	5701	6	69.0	5801	11	73.0
5502	3	57.5	5602	0	18.5	5702	8	71.5	5818	0	18.5
5503	2	50.0	5617	0	18.5	5703	2	50.0	5842	0	18.5
5517	0	18.5	5618	0	18.5	5742	5	66.0	5843	7	70.0
5518	3	57.5	5619	0	18.5	5743	5	66.0	5845	0	18.5
5519	3	57.5	5642	3	57.5	5744	0	18.5	5859	2	50.0
5542	3	57.5	5643	2	50.0	5745	8	71.5	5886	4	62.5
5543	0	18.5	5644	0	18.5	5746	3	57.5	5889	15	75.0
5544	0	18.5	5645	0	18.5	5747	5	66.0	5890	12	74.0
5550	0	18.5	5646	1	41.5	5749	1	41.5			
5551	1	41.5	5649	1	41.5	5751	1	41.5			
5552	0	18.5	5650	2	50.0	5752	0	18.5			
5553	0	18.5	5652	0	18.5	5753	0	18.5			
5554	0	18.5	5655	0	18.5	5754	2	50.0			
5555	0	18.5	5656	0	18.5	5755	1	41.5			
5556	0	18.5	5657	0	18.5	5756	0	18.5			
5558	0	18.5	5687	0	18.5	5757	0	18.5			
5559	0	18.5	5692	0	18.5	5758	1	41.5			
5591	0	18.5	5693	1	41.5	5785	1	41.5			
5598	1	41.5	5694	0	18.5	5786	3	57.5			
			5695	0	18.5	5793	4	62.5			
			5696	0	18.5	5798	0	18.5			
			5697	3	57.5	5799	5	66.0			
Mean Value	0.9	31.8		0.8	29.7		2.7	46.6		5.7	51.1

[a]Six hours/day, five days/week, 62 days.

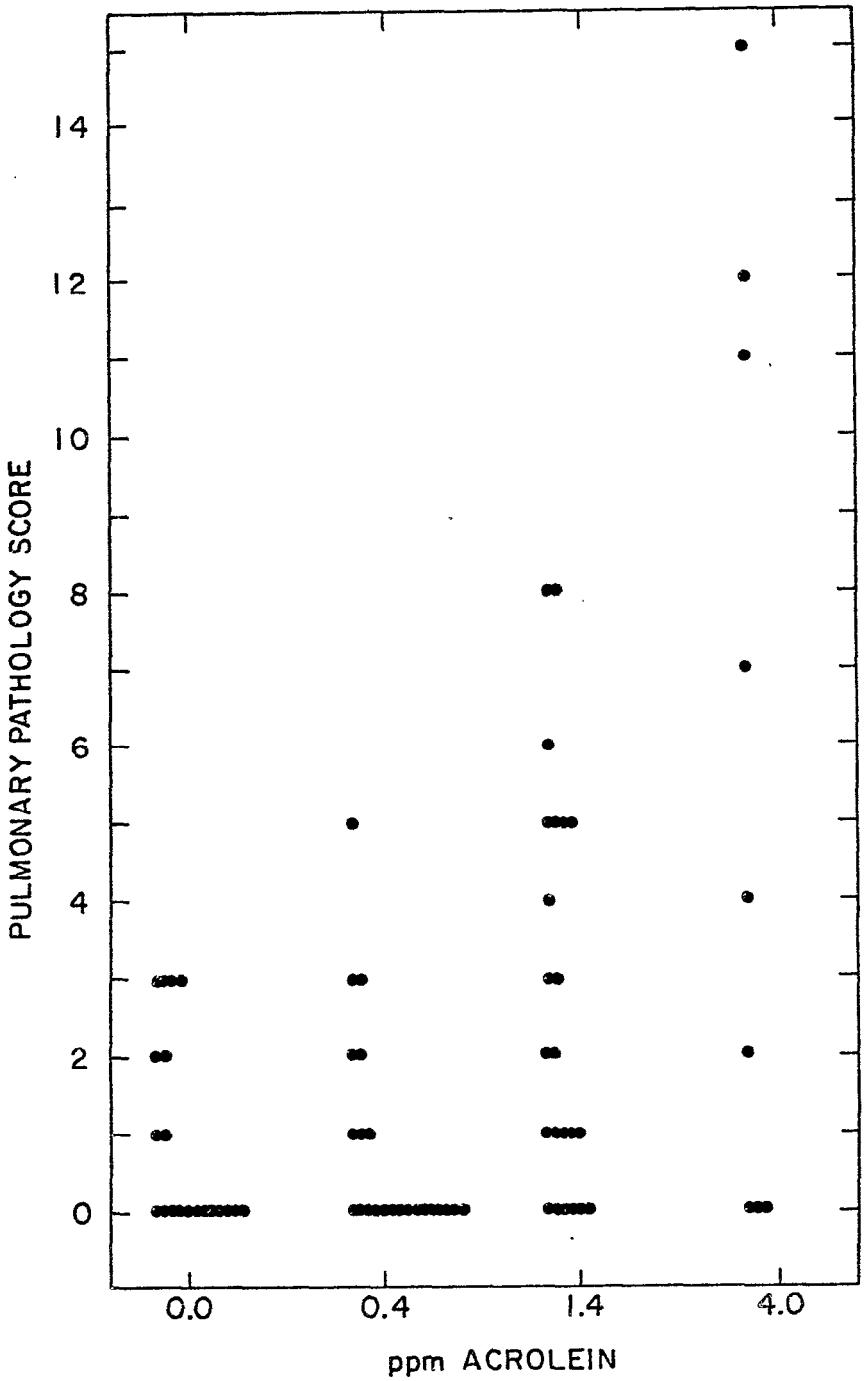

Figure 15: Frequency of pulmonary pathology scores of Fischer 344 rats exposed to acrolein for 62 days (6 hours/day, 5 days/week). (See text for details.)

Lung Composition Data

The right lungs of animals which completed pulmonary function tests were assayed for protein, DNA, elastin, and hydroxyproline (an index of collagen) concentration as well as water content. The mean animal weight of each exposure group has been provided in Table 14. The data for individual animals has been provided in Appendix F. The relationship of these data to the pathology and the pulmonary function data of all exposure groups was also statistically evaluated (see Statistical Relationships Among Data).

Lung Weight and Water Content. Although the body weights of the rats exposed to 4.0 ppm acrolein were markedly reduced, the lungs of these animals were significantly heavier than those of any other exposure group (Table 14). This increased lung weight was due to a 20% increase in dry weight mass accompanied by a 1.5% increase in water content per unit dry weight (Table 14).

Lung DNA. The total lung DNA content of the 4.0 ppm animals was significantly greater than that of the other exposure groups (Table 15). However, if expressed on a per gram wet weight basis, the DNA concentration of this group was less than that of the control or 0.4 ppm exposure groups. The marked increase in lung dry weight in the 4.0 ppm group (Table 14) and the absence of a significant difference in the amount of DNA per unit dry weight (Table 15) were indications that the increased lung weight of the high dose group was in part due to increased cellularity.

Lung Protein. The concentration of lung protein followed a pattern very similar to that observed for pulmonary DNA in all exposure groups. The uniform concentration of protein per milligram dry weight and per

milligram DNA (Table 16) again indicated increased lung tissue in the 4.0 ppm exposed animals.

Lung Elastin. The total lung elastin content of rats exposed to 4.0 ppm acrolein was twice that of the control and lower exposure groups (Table 17). This marked increase was also evident when the elastin concentration was based on DNA, protein, or dry weight (Table 17). However, such an increase in elastin content would have little effect on total lung weight because elastin accounts for less than 1% of lung wet weight.

Lung Collagen. Hydroxyproline was used as an index of lung collagen content. Of the lung constituents assessed, only collagen content changed significantly from control values in the animals exposed to 1.4 ppm acrolein. Lung hydroxyproline content was increased by exposure to 1.4 ppm as well as 4.0 ppm acrolein (Table 18). The total lung hydroxyproline content of the 4.0 ppm group was significantly greater than that of all other exposure groups while the 1.4 ppm group had significantly more hydroxyproline than the control and low dose animals but significantly less than the high dose group. When based on wet weight, the pulmonary hydroxyproline concentration of the 1.4 and 4.0 ppm groups did not differ, and both were significantly greater than the control concentration (Table 18). When based on either dry weight or protein content, the hydroxyproline concentration of the 4.0 ppm lungs was greater than that of all other exposure groups, and the hydroxyproline concentration of the 1.4 ppm lungs was markedly increased when compared to that of the controls and 0.4 ppm exposed lungs (Table 18). Although these changes were rather marked, increased collagen content contributes little to total lung weight because of the small amount present.

Table 14. Body Weight and Lung Weight Data from Fischer 344 Rats Exposed to Filtered Air or Acrolein[a]

	ACROLEIN EXPOSURE CONCENTRATION (ppm)			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=22	n=9
Body Wt.(gms)	326.1(2.66)[b]	336.9(4.49)	330.5(3.64)	241.0(3.06)[c]
Lung Wt.(gms)	1.29(.025)	1.28(.029)	1.35(.039)	1.71(.055)[c]
Total Dry Wt.(mg)	267.9(5.49)	263.1(7.12)	276.1(8.16)	324.0(10.14)[c,d]
% Dry Wt.	20.7(.19)	20.8(.24)	20.6(0.26)	19.2(0.47)[c,d]

[a] Six hours/day, five days/week, 62 days.

[b] Mean(±s.e.).

[c] Significantly different ($p<0.0083$) from all other exposure groups by Bonferroni multiple comparison technique.

[d] n=8.

Table 15. Lung DNA Data from Fischer 344 Rats Exposed to Filtered Air or Acrolein[a]

	ACROLEIN EXPOSURE CONCENTRATION (ppm)			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=22	n=8
Total Lung DNA (mg)	6.24(.114)[b]	6.21(.141)	6.22(.121)	7.28(.136)[c]
mg DNA/gm Wet Wt.	4.83(.049)	4.85(.055)	4.65(.083)	4.32(.104)[d]
mg DNA/mg Dry Wt.	0.023(.000)	0.024(.000)	0.023(.000)	0.023(.000)
mg DNA/mg Protein	0.042(.000)	0.043(.000)	0.041(.001)	0.041(.001)

[a]Six hours/day, five days/week, 62 days.

[b]Mean (±s.e.).

[c]Significantly different ($p<0.0083$) from all other exposure groups by Bonferroni multiple comparison technique.

[d]Significantly different ($p<0.0083$) from the 0.0 and 0.4 ppm groups by Bonferroni multiple comparison technique.

Table 16. Lung Protein Data from Fischer 344 Rats Exposed to Filtered Air or Acrolein[a]

	ACROLEIN EXPOSURE CONCENTRATION (ppm)			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=22	n=8
Total Lung Protein (mg)	147.5(2.97)[b]	146.5(3.82)	154.5(4.71)	177.7(5.25)[c]
mg Protein/gm Wet Wt.	114.1(.75)	114.2(1.37)	114.7(1.65)	105.3(2.53)[c]
mg Protein/mg Dry Wt.	0.55(.005)	0.56(.009)	0.56(.006)	0.55(.008)
mg Protein/mg DNA	23.7(0.23)	23.6(.23)	24.8(.52)	24.4(.54)

[a]Six hours/day, five days/week, 62 days.

[b]Mean (±s.e.).

[c]Significantly different ($p<0.0083$) from all other exposure groups by Bonferroni multiple comparison technique.

Table 17. Lung Elastin Data from Fischer 344 Rats Exposed to Filtered Air or Acrolein[a]

	ACROLEIN EXPOSURE CONCENTRATION (ppm)			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=22	n=8
Total Lung Elastin (mg)	5.91(.126)[b]	6.10(.136)	6.43(.169)	12.53(1.028)[c]
mg Elastin/gm Wet Wt.	4.60(.125)	4.80(.129)	4.84(.173)	7.39(.558)[c]
mg Elastin/mg Dry Wt.	0.02(.001)	0.02(.001)	0.02(0.001)	0.04(.003)[c]
mg Elastin/mg DNA	0.95(.025)	0.99(.024)	1.04(.030)	1.71(.124)[c]
mg Elastin/mg Protein	0.04(.001)	0.04(.001)	0.04(.001)	0.07(.006)[c]

[a]Six hours/day, five days/week, 62 days.

[b]Mean (±s.e.).

[c]Significantly different ($p<0.0083$) from all other exposure groups by Bonferroni multiple comparison technique.

Table 18. Lung Hydroxyproline Data from Fischer 344 Rats Exposed to Filtered Air and Acrolein[a]

	ACROLEIN EXPOSURE CONCENTRATION (ppm)			
	0.0	0.4	1.4	4.0
	n=24	n=23	n=22	n=8
Total Lung Hydroxyproline (mg)	2.38(.043)[b]	2.53(.052)	2.82(.054)[c]	3.99(.147)[c]
mg OH-PRO/gm Wet Wt.	1.85(.032)	1.99(.053)	2.11(.058)[d]	2.36(.077)[e]
mg OH-PRO/mg Dry Wt.	0.009(.000)	0.010(.000)	0.010(.000)[f]	0.012(.000)[c]
mg OH-PRO/mg DNA	0.38(.007)	0.41(.010)	0.45(.010)[c]	0.55(.017)[c]
mg OH-PRO/mg Protein	0.016(.000)	0.017(.000)	0.018(.000)[d]	0.023(.001)[c]

[a] Six hours/day, five days/week, 62 days.

[b] Mean (±s.e.).

[c] Significantly different ($p<0.0083$) from all other exposure groups by Bonferroni multiple comparison technique.

[d] Significantly different ($p<0.0083$) from 0.0 ppm group by Bonferroni multiple comparison technique.

[e] Significantly different ($p<0.0083$) from 0.0 and 0.4 ppm groups by Bonferroni multiple comparison technique.

[f] Significantly different ($p<0.0083$) from 0.0 and 4.0 ppm groups by Bonferroni multiple comparison technique.

Cytology Results

Bone Marrow. The results of the sister chromatid exchange (SCE) and cellular proliferation studies in populations of bone marrow cells from control, 0.4, 1.4, and 4.0 ppm acrolein-exposed rats have been provided in Table 19. The SCE data were normalized by square root transformation and were then compared using the Student's t test. No significant differences were observed between any of the exposure groups and the controls. No significant changes were observed between the control and exposed animals in the relative proportions of first-, second-, and third-generation metaphase cells (cell proliferation kinetics, Table 19). Analysis of chromosomal aberrations was not possible, because few first-generation metaphase cells were observed in the bone marrow of rats sacrificed after 25 hours of BrdUrd infusion.

Peripheral Blood Lymphocytes. The analysis of the SCE and chromosomal aberration data from peripheral blood lymphocytes of control rats and those exposed to acrolein have been provided in Tables 20 and 21. Using a square root transformation to analyze the SCE data for statistical differences (Student's t-test), no significant differences were observed between the control cell populations and those of any exposure group (Table 20). Analysis of the relative proportions of first-, second-, and third-generation metaphase cells indicated that there were no differences in the cell proliferation kinetics among these groups (Table 20). Because the number of first-generation metaphase cells was limited, scoring of chromosomal aberrations was restricted to 50 cells (first-generation) per animal. On the basis of these data, there were no statistically significant differences (Student's t-test) in the frequency of chromosomal aberrations or in the percent abnormal cells between the control rats and rats exposed to either 1.4 or 4.0 ppm acrolein (Table 21). Slides from the 0.4 ppm exposed animals

were not scored because the higher exposure concentrations did not demonstrate an effect.

Lung Alveolar Macrophages. The studies on alveolar macrophages from lung washes were severely limited by both a very low mitotic index and the presence of only first-generation cells in all exposure groups. Consequently, it was not possible to score for SCEs or to score chromosomal aberrations.

Sperm Morphology. Sperm samples from at least nine rats in each exposure group were examined for abnormal cells. The data from individual animals are available in Appendix G. There were no significant differences among groups when the data were analyzed by either the Kruskall-Wallis non-parametric test or by one way ANOVA after arcsin transformation of the data.

Table 19. Frequency of Sister Chromatid Exchange and Relative Rates of Cell Proliferation in Bone Marrow Cells of Fischer 344 Rats Exposed to Acrolein[a]

Acrolein Concentration (ppm)	Animal Number	SCE/Cell[b]		Cell Proliferation Kinetics (%)[c]		
		Raw Data	$\sqrt{x}$ Transformed Data	I	II	III
0.0	5504	5.52(0.32)	2.31(0.08)	23	70	7
	5506	4.89(0.59)	2.48(0.12)	34	64	2
	5508	5.12(0.41)	2.22(0.09)	27	72	1
	5520	5.56(0.37	2.32(0.08)	1	66	33
	5584	5.00(0.36)	2.20(0.08)	12	77	11
	$\sum$[d]	5.22(0.15)	2.31(0.06)	19,4(6,54)	69.8(2.56)	10.8(6.52)
0.4	5604	5.48(0.39)	2.30(0.09)	8	91	1
	5605	5.56(0.37)	2.33(0.08)	22	76	2
	5606	5.60(0.34)	2.34(0.07)	14	84	2
	5607	4.60(0.36)	2.10(0.09)	24	76	0
	5608	5.56(0.44)	2.31(0.10)	16	83	1
	$\sum$	5.36(0.21)	2.28(0.05)	16.8(3.21)	82.0(3.14)	1.2(0.42)
1.4	5704	5.32(0.52)	2.23(0.18)	10	87	3
	5705	5.40(0.46)	2.27(0.10)	12	84	4
	5706	5.44(0.56)	2.27(0.11)	65	35	0
	5707	5.40(0.51)	2.26(0.11)	15	83	2
	5708	5.20(0.48)	2.22(0.11)	21	69	10
	$\sum$	5.35(0.05)	2.25(0.01)	24.6(11.48)	71.6(10.80)	3.8(1.88)

Table 19 -- Continued

Acrolein Concentration (ppm)	Animal Number	SCE/Cell[b]		Cell Proliferation Kinetics (%)[c]		
		Raw Data	$\sqrt{x}$ Transformed Data	I	II	III
4.0	5805	5.12(0.38)	2.22(0.09)	19	71	10
	5806	4.16(0.24)	2.02(0.06)	13	80	7
	5808	5.12(0.51)	2.20(0.11)	32	67	1
	5884	5.88(0.44)	2.37(0.10)	26	73	1
	5820	4.64(0.32)	2.12(0.07)	2	79	19
	$\sum^{d}$	4.98(0.32)	2.19(0.06)	18.4(5.82)	74.0(2.74)	7.6(3.73)

[a]Six hours/day, five days/week, 62 days.

[b]Mean frequency of SCE/cell (±s.e.) among (n=25) cells for each animal.

[c]Percent of cells which had replicated for one (I), two (II), or three (III) generations in a sample of 100 randomly chosen metaphase cells.

[d]Mean of mean frequency of SCE/cell (±s.e.) among n animals and mean percent of metaphase cells in each generation (±s.e.) among n animals.

Table 20. Frequency of Sister Chromatid Exchange and Relative Rates of Cell-Proliferation in PHA-Stimulated Peripheral Blood Lymphocytes of Fischer 344 Rats Exposed to Acrolein[a]

Acrolein Concentration (ppm)	Animal Number	SCE/Cell[b]		Cell Proliferation Kinetics (%)[c]		
		Raw Data	√Root Transformed	I	II	III
0.0	5504	8.52(0.52)	2.87(0.11)	39	30	31
	5505	8.31(0.44)	2.81(0.09)	46	44	10
	5508	7.73(0.46)	2.77(0.08)	51	35	14
	5521	7.32(0.39)	2.68(0.09)	41	53	6
	$\sum$[d]	7.97(0.32)	2.78(0.05)	44.25(3.10)	40.5(5.86)	15.25(6.35)
0.4	5604	8.20(0.75)	2.82(0.10)	41	32	27
	5646	6.64(0.46)	2.49(0.09)	44	37	19
	5606	7.76(0.57)	2.74(0.10)	45	38	17
	5607	8.84(0.56)	2.94(0.10)	50	45	5
	$\sum$	7.86(0.53)	2.75(0.11)	45.0(2.16)	38.0(3.09)	17.0(5.25)
1.4	5707	8.00(0.64)	2.78(0.10)	46	28	26
	5708	8.08(0.67)	2.79(0.11)	43	40	17
	5746	6.80(0.47)	2.57(0.08)	42	34	24
	5704	8.56(0.48)	2.90(0.08)	44	50	6
	$\sum$	7.86(0.43)	2.76(0.08)	43.8(0.99)	38.0(5.42)	18.2(5.21)

Table 20 - Continued

Acrolein Concentration (ppm)	Animal Number	SCE/Cell[b]		Cell Proliferation Kinetics (%)[c]		
		Raw Data	√Root Transformed	I	II	III
4.0	5801	7.64(0.36)	2.74(0.07)	47	37	16
	5845	7.92(0.50)	2.78(0.09)	47	45	8
	5805	8.04(0.61)	2.78(0.11)	43	25	32
	5806	8.60(0.53)	2.90(0.09)	40	40	20
	$\sum$	8.05(0.23)	2.80(0.04)	44.3(1.96)	36.8(4.91)	19.0(5.77)

[a]Six hours/day, five days/week, 62 days.

[b]Mean frequency of SCE/Cell (±s.e.) among (n=25) cells.

[c]Percent of cells which had replicated for one (I), two (II), or three (III) generations in a sample of 100 randomly chosen metaphase cells.

[d]Mean of mean frequency of SCE/Cell (±s.e.) among n animals.

Table 21. Chromosomal Aberration Frequencies in PHA-Stimulated Peripheral Blood Lymphocytes of Fischer-344 Rats Exposed to Acrolein[a]

Acrolein Concentration (ppm)	Animal Number	Cells Scored	Chromatid Aberrations: Achromatic Lesions	Chromatid Aberrations: Deletions	Chromatid Aberrations: Exchanges	Chromosome Aberrations	Abnormal Cells (%)
0.0	5504	50	3	0	0	0	6.0
	5505	50	3	2	0	0	10.0
	5521	50	2	2	0	0	6.0
	5508	50	1	1	0	0	4.0
	Animal variability[b]	(n=4)	2.25(0.55)	1.25(0.55)			6.5(1.45)
1.4	5707	50	3	2	0	0	8.0
	5708	50	2	3	0	0	10.0
	5746	50	1	1	0	0	4.0
	5704	50	4	0	0	0	6.0
	Animal variability		2.5 (0.75)	1.5 (0.75)			7.0 (1.49)
4.0	5801	50	2	1	0	0	6.0
	5805	50	4	3	0	0	12.0
	5806	50	1	0	0	0	2.0
	5845	50	2	4	0	0	6.0
	Animal variability		2.25 (0.73)	2.0 (1.05)			6.5 (2.38)

[a] Six hours/day, five days/week, 62 days.

[b] Statistical analysis based on animal-to-animal variability, assuming a normal distribution. Mean (±standard error).

Reproductive Potential Studies

The reproductive fitness data resulting from the mating of acrolein exposed and control male rats to unexposed females has been provided in Table 22. No significant differences (Student's t-test) were observed between females mated with control males and females mated with males from any of the acrolein exposure groups. The reproductive potential of female rats appeared unaffected by exposure to acrolein (Table 23).

Table 22 Reproductive Fitness of Male Fischer 344 Rats After Exposure to 0.4, 1.4, or 4.0 ppm Acrolein[a]. Each Male Was Caged with Two Unexposed Female Rats Beginning Six Days After the Final Exposure.

	Acrolein Exposure Concentration (ppm)			
	0.0	0.4	1.4	4.0
Pregnant Females/ Number Mated	7/16	6/16	8/14	5/10
Corpora lutea	10.1(1.16)[b]	10.8(0.48)	11.6(0.65)	10.8(1.11)
Viable Embryos	9.0(1.07)	10.2(0.65)	9.0(0.89)	9.0(1.05)
Early Deaths	0.1(0.14)	0.0	0.4(0.18)	0.0
Late Deaths	0.0	0.0	0.0	0.0
Preimplantation Losses	1.0(0.58)	0.7(0.33)	2.2(0.53)	1.8(0.38)

[a]Six hours/day, five days/week, 62 days.

[b]Mean (± s.e.)

Table 23. Reproductive Fitness of Female Fischer 344 Rats After Exposure to 0.4, 1.4, and 4.0 ppm Acrolein[a]. Each Female was Mated with a Single Proven Male Beginning Six Days After the Final Exposure

	Acrolein Exposure Concentration (ppm)			
	0.0	0.4	1.4	4.0
Pregnant Females/ Number Mated	6/8	4/8	8/8	5/8
Corpora lutea	10.5(0.72)[b]	8.5(2.22)	11.2(0.16)	10.4(0.60)
Viable Embryos	8.8(0.48)	7.8(2.29)	9.8(0.36)	9.4(0.51)
Early Deaths	0.2(0.16)	0.0	0.8(0.31)	0.0
Late Deaths	0.2(0.16)	0.2(0.25)	0.0	0.0
Preimplantation Losses	1.3(0.96)	0.5(0.29)	0.8(0.25)	1.0(0.45)

[a]Six hours/day, five days/week, 62 days.

[b]Mean (± s.e.).

Statistical Relationships Among Data

Correlation Analysis. Twenty-six variables from each animal were available for correlation analysis after pulmonary function testing, assessment of lung connective tissue, and histopathologic examination of rats in each designated subgroup. The variables utilized in these analyses are listed in Table 24. Many of the respiratory physiology parameters have been expressed as a function of either FRC_d, TLC_d, or VC, on which they were dependent. All of the lung composition data were normalized to dry weight. Only those sets of correlation coefficients where at least one exposure group demonstrated a significant linear association between the parameters considered have been provided in Table 25.

Several significant associations were found to exist between functional and compositional variables in the control group. Elastin concentration was associated with FRC_b, hydroxyproline content with f, lung weight with $\dot{V}_E$, and protein with PEF, EFR_{50}, and $\dot{V}_E$. While large normal lungs have greater volumes and probably greater maximum flows than small normal lungs there is no reason to speculate that these relationships would be maintained after pulmonary insult. In fact, the above associations were not evident in any of the acrolein exposure groups. The control pathology ranking, which probably reflected occasional low level infective pneumonitis, was significantly associated with maximal flows of MEFV.

The majority of the significant correlations between parametric data occurred in the 4.0 ppm group. The directional characteristics of these correlations reflected an association between the proliferation or accumulation of connective tissue and disrupted flow in the airways, and to an extent the reduced compliance: elastin to EFR_{25}, -0.895; to ΔEFR_{25},

-0.903; to R_{us}, 0.802; and to RV, 0.707; hydroxyproline to RV, 0.826; to C_{DYN}, -0.748; and lung weight to RV, 0.752.

Significant rank correlations of pathology with either functional or compositional indices were concentrated in the 1.4 and 4.0 ppm groups (Table 25). Generally with increasing lung damage, maximum flows during the forced volume effort (ΔEFR_{25}) were diminished while lung volumes increased (RV, FRC_b). Minute volumes were inversely correlated with the pathology indices within the 4.0 ppm group, indicating a fall in ventilation with increased severity of the acrolein induced lesion. The $DLCO_{sb}$ of the 1.4 ppm group was inversely correlated with the pathology scores. This inverse relationship was a consistent finding except in the case of the 0.4 ppm animals. Significant inverse associations were found between the pathology index and elastin as well as hydroxyproline concentration in the 1.4 ppm group (Table 25). Although not significant, the inverse relationship was evident in the control and low dose groups. However, a positive correlation was demonstrated in the 4.0 ppm animals, statistically significant in the case of elastin.

Discriminant Analysis. Stepwise discriminant analysis was used to identify those normalized pulmonary function and lung composition parameters which best distinguished the four exposure groups. This technique selected and linearly combined a set of discriminating variables which forced the exposure groups to be as distinct as possible. When completed, the effectiveness of the derived discriminating function was checked by using it to classify the animals originally studied.

All of the lung composition data used in these analyses were expressed as a function of dry lung weight (Table 24). When stepwise discriminant analysis was applied to data from all exposure groups, hydroxyproline,

elastin, and DNA were required for discrimination of the groups; hydroxyproline had the greatest discriminating power. The first discriminating function based on these variables explained 83.9% of the linear dispersion of the exposure groups. When this classification function was used to categorize the animals, 57% were correctly classified (Table 26). When the same analysis was applied to the respiratory physiology data (Table 26) $DLCO_{sb}$, FRC_b, and EFR_{25} were the most discriminating variables. The first canonical variables explained 91.4% of the linear dispersion of the groups. The classification function based on these variables correctly classified 57.5% of the cases (Table 26). If both the respiratory physiology and lung composition data were used in the stepwise discriminant analysis, 65.3% of the animals were correctly placed with the resulting classification function (Table 26). The discriminating variables in this case were FRC_b, $DLCO_{sb}$, EFR_{25}, and hydroxyproline. The dispersion of the individual animals, as well as the group centroids, based on the first and second discriminant functions (canonical variables) is illustrated in Appendix H.

Because of the marked differences observed in the 4.0 ppm animals, relative to the other exposure groups, the same analyses and classification procedures were conducted but with the high dose group deleted. When the analysis was limited to the biochemical parameters of the 0.0, 0.4, and 1.4 ppm exposure groups, hydroxyproline and DNA proved to be the most discriminating. The first canonical variable explained 91.8% of the linear dispersion among the three groups and the classification function correctly grouped 55.1% of the rats (Table 27). Stepwise discriminant analysis on normalized respiratory physiology data from these three groups found EFR_{25} to be the most discriminating parameter. The first discriminant function based on this variable explained 100% of the linear dispersion of the groups. However, the resulting classification function properly classified only

53.1% of the animals in these three exposure groups (Table 27). EFR_{25}, hydroxyproline, and DNA surfaced as the most discriminating variables when both respiratory physiology and lung composition data were used in the analysis. The first discriminating function based on these variables explained 64% of the linear dispersion among the groups and the second discriminating function accounted for the other 36%. The dispersion of the individual animals in these three groups based on these discriminant functions is illustrated in Appendix H. Sixty-seven percent of the animals were successfully categorized with this classification function (Table 27).

Stepwise discriminant analysis was also performed on the data from the possible pair combinations of the control, 0.4, and 1.4 ppm exposure groups to determine which parameters were most discriminating between the groups. The results of these analyses and the success of the resulting classification functions in placing the test animals into their respective groups have been presented in Table 28. Hydroxyproline concentration consistently appeared as the most discriminating lung composition variable either alone or in conjunction with DNA. When the control and 1.4 ppm groups were compared using respiratory physiology data only, no combination of the measured parameters provided significant linear dispersion between the groups. When the control and 1.4 ppm groups were compared on the lung composition data alone or in conjunction with the respiratory function parameters, hydroxyproline and DNA appeared as the discriminating variables. The slightly different success rate in classifying these animals (Table 28) was due to the difference in sample size; if the data set for an animal was incomplete it could not be used in the analysis. Of the pulmonary function variables, EFR_{25} consistently provided significant linear dispersion between the 0.4 and 1.4 ppm exposure groups.

Table 24. Variables Used in the Pearson and Spearman Correlations and the Discriminant Analysis of Pulmonary Function, Lung Composition, and Pathology Data.

PULMONARY FUNCTION PARAMETERS

UNNORMALIZED

1. Heart rate (HR)
2. Tidal volume (V_T)
3. Transpulmonary pressure (P_L)
4. Frequency of breathing (f)
5. Minute volume ($\dot{V}_E$)
6. Functional reserve capacity (FRC_b)
7. Upstream airway resistance (R_{us})

NORMALIZED TO FUNCTIONAL RESERVE CAPACITY DETERMINED BY DILUTION (FRC_d)

1. Resistance (R_L)
2. Dynamic compliance (C_{DYN})
3. Quasi-static compliance determined by chord slope (QSC_{cs})
4. Quasi-static compliance determined by steep slope (QSC_{ss})

NORMALIZED TO TOTAL LUNG CAPACITY DETERMINED BY DILUTION (TLC_d)

1. Expiratory reserve volume (ERV)
2. Inspiratory capacity (IC)
3. Diffusing capacity for CO measured by rebreathing ($DLCO_{rb}$)
4. Diffusing capacity for CO measured by single breath ($DLCO_{sb}$)
5. Trapped gas (FRC_b-FRC_d)
6. Residual volume (RV)

NORMALIZED TO VITAL CAPACITY (VC)

1. Peak expiratory flow (PEF)
2. Expiratory flow rate at X% vital capacity, where X = 10, 25, or 50 (EFR_X)
3. Difference in the flow at 25% vital capacity above or below that volume estimated by a chord slope drawn from EFR_{50} to EFR_0 (ΔEFR_{25})

LUNG COMPOSITION PARAMETERS

NORMALIZED TO DRY WEIGHT

1. Lung weight
2. Protein
3. Elastin
4. Collagen

PATHOLOGY

Scores (Table 13) were ranked and used only in the Spearman correlations.

Table 25. Pearson Correlation Coefficients of Pulmonary Physiology vs. Lung Composition Data and Spearman Rank Correlation Coefficients of Ranked Pathology Scores vs. Ranked Pulmonary Physiology and Lung Composition Data[a]

	ppm Acrolein	Lung Weight	Elastin	Hydroxyproline	Protein	DNA	Pathology Rank[b]
V_T	0.0				-0.0254		
	0.4				0.1466		
	1.4				-0.4473[c]		
	4.0				0.0636		
P_L							
f	0.0				0.4471[c]		
	0.4				-0.3515		
	1.4				0.0507		
	4.0				0.2687		
R_L	0.0			0.0156			
	0.4			0.4488[c]			
	1.4			-0.2025			
	4.0			-0.2655			
C_{DYN}	0.0			0.0890			
	0.4			-0.1512			
	1.4			-0.0779			
	4.0			-0.7481[c]			
ERV							
HR							

Table 25 -- continued

	ppm Acrolein	Lung Weight	Elastin	Hydroxyproline	Protein	DNA	Pathology Rank[b]
IC	0.0						-0.4805[c]
	0.4						0.2538
	1.4						0.1152
	4.0						-0.3172
FRC_b	0.0		0.4126[c]				-0.2822
	0.4		0.2527				-0.1002
	1.4		0.2742				-0.5101[c]
	4.0		0.3725				0.5611
$DLCO_{sb}$	0.0						-0.1719
	0.4						0.3603
	1.4						-0.6061[c]
	4.0						-0.2196
$DLCO_{rb}$	0.0						-0.0647
	0.4						0.1781
	1.4						-0.4717[c]
	4.0						0.1464
QSC_{ss}							
QSC_{cs}							
PEF	0.0				0.4119[c]		0.4912[c]
	0.4				0.3746		0.0369
	1.4				-0.2704		0.3972
	4.0				0.3247		-0.1220

Table 25 -- continued

	ppm Acrolein	Lung Weight	Elastin	Hydroxyproline	Protein	DNA	Pathology Rank[b]
EFR_{50}	0.0				0.5606[c]		0.5239[c]
	0.4				0.3818		0.0402
	1.4				-0.2796		0.4494[c]
	4.0				0.0607		-0.3660
EFR_{25}	0.0		-0.2995				0.4175[c]
	0.4		-0.0551				-0.1752
	1.4		-0.0018				0.3680
	4.0		-0.8955[c]				-0.6343
EFR_{10}							
ΔEFR_{25}	0.0		-0.2067				0.1179
	0.4		-0.0547				-0.3314
	1.4		0.0139				0.1828
	4.0		-0.9028[c]				-0.8295[c]
R_{us}	0.0		0.1452				
	0.4		0.3465				
	1.4		-0.0222				
	4.0		0.8023[c]				
RV	0.0	-0.0018	0.0064	0.0138			0.0925
	0.4	-0.1373	-0.1600	-0.3671			-0.3667
	1.4	0.0053	0.1670	0.2634			-0.1767
	4.0	0.7515[c]	0.7067[c]	0.8257[c]			0.8295[c]
VE	0.0	0.4645[c]			0.4238[c]		-0.0287
	0.4	-0.2903			-0.2459		0.0192
	1.4	-0.1828			-0.0787		-0.0791
	4.0	-0.6649			0.2799		-0.8295[c]

Table 25 -- continued

	ppm Acrolein	Lung Weight	Elastin	Hydroxyproline	Protein	DNA	Pathology Rank[b]
Pathology[b] Rank	0.0		-0.2169	-0.0401		0.0696	
	0.4		-0.2763	-0.1444		-0.4395[c]	
	1.4		-0.4471[c]	-0.4509[c]		0.1613	
	4.0		0.8051[c]	0.4636		-0.1220	

[a]Only sets of correlation coefficients where at least one association was statistically significant ($p<0.05$) are listed.

[b]Ranked data analyzed using Spearman Rank Correlation.

[c]Significant linear association ($p<0.05$).

Table 26. Categorization of Fischer 344 Rats Exposed to 0.0, 0.4, 1.4, or 4.0 ppm Acrolein by a Classification Function Derived from Stepwise Discriminant Analysis of Selected Parameters

Lung Composition Data

	Number of Cases Classified into Group				Percent Correct
Group	0.0	0.4	1.4	4.0	
0.0	17	4	3	0	70.8
0.4	8	7	8	0	30.4
1.4	1	6	15	0	68.2
4.0	0	0	3	5	62.5
Total	26	17	29	5	57.1

Pulmonary Function Data

	Number of Cases Classified into Group				Percent Correct
Group	0.0	0.4	1.4	4.0	
0.0	13	4	4	0	61.9
0.4	4	16	3	0	69.6
1.4	8	6	6	0	30.0
4.0	1	0	1	7	77.8
Total	26	26	14	7	57.5

Lung Composition and Pulmonary Function Data

	Number of Cases Classified into Group				Percent Correct
Group	0.0	0.4	1.4	4.0	
0.0	16	3	2	0	76.2
0.4	3	13	7	0	56.2
1.4	3	5	12	0	60.0
4.0	0	0	2	6	75.0
Total	22	21	23	6	65.3

Table 27. Categorization of Fischer 344 Rats Exposed to 0.0, 0.4, or 1.4 ppm Acrolein by a Classification Function Derived from the Discriminant Variables Defined by Stepwise Discriminant Analysis of Selected Parameters

Lung Composition Data

	Number of Cases Classified into Group			Percent Correct
	0.0	0.4	1.4	
Group				
0.0	17	6	1	70.8
0.4	7	7	9	30.4
1.4	2	6	14	63.6
Total	26	19	24	55.1

Pulmonary Function Data

	Number of Cases Classified into Group			Percent Correct
	0.0	0.4	1.4	
Group				
0.0	11	5	5	52.4
0.4	3	17	3	73.9
1.4	7	7	6	30.0
Total	21	29	14	53.1

Lung Composition and Pulmonary Function Data

	Number of Cases Classified into Group			Percent Correct
	0.0	0.4	1.4	
Group				
0.0	16	2	3	76.2
0.4	5	13	5	56.5
1.4	2	4	14	70.0
Total	23	19	21	67.2

Table 28. Discriminating Variables Determined by Stepwise Discriminant Analysis and Correct Categorization of Animals with the Resulting Classification Function After Analysis of Selected Data Sets from Rats Exposed to 0.0, 0.4, or 1.4 ppm Acrolein

Variable \ Groups		0.0 vs. 0.4	0.0 vs. 1.4	0.4 vs. 1.4
Lung Composition Parameters Only	% Correct Classification (n)	75.0 (24) 69.6 (23)	95.8 (24) 59.1 (22)	87.0 (23) 50.0 (22)
	Discriminating Variable(s)	Hydroxyproline DNA	Hydroxyproline DNA	Hydroxyproline DNA
Respiratory Physiology Parameters Only	% Correct Classification (n)	81.0 (21) 82.6 (23)		91.3 (23) 30.0 (20)
	Discriminating Variable(s)	$DLCO_{sb}$ R_L EFR_{25}	None[a]	EFR_{25}
Lung Composition and Respiratory Physiology Parameters	% Correct Classification (n)	81.0 (21) 82.6 (23)	100 (21) 65.0 (20)	91.3 (23) 30.0 (20)
	Discriminating Variable(s)	$DLCO_{sb}$ R_L EFR_{25}	Hydroxyproline DNA	EFR_{25}

[a]None of the variables provided significant discriminating power.

DISCUSSION

The mortality observed in the 4.0 ppm chamber predominantly occurred during the first three weeks of exposure. A similar mortality pattern was reported for Wistar rats exposed to 4.9 ppm acrolein (32) (6 hour/day, 5 days/week) and Sprague-Dawley derived animals exposed to 4.0 ppm acrolein (33). None of the female Fischer 344 rats exposed to 4.0 ppm acrolein died, although they rapidly lost weight and remained at less than their starting weights throughout the exposure period. The change in rate of weight gain observed here was in agreement with other studies where rats were repeatedly exposed to similar acrolein concentrations (32-34).

When reviewing the organ-to-body weight ratios, it must be remembered that animals were necropsied six days after the final exposure, during which time the 4.0 ppm group gained a considerable amount of weight. Therefore, the recorded organ-to-body weight ratios of the high dose group were probably different than they would have been if the animals were sacrificed immediately after exposures were terminated. The significantly greater absolute lung weight of the 4.0 ppm rats was attributed to increased cellularity. Although severe focal edema was observed in these lungs, the water content was only 1.5% greater than that of control lungs. The increased lung-to-body weight ratio was the only changed ratio in the 4.0 ppm group directly attributable to acrolein exposure. Although changes have been reported in the liver-to-body weight ratio of rats after exposure to acrolein (33,35), such changes were not recorded here. Feron *et al.* (32) did not observe changes in the liver-to-body weight ratios of rats of comparable weight to those in this study, repeatedly exposed to 4.9 ppm acrolein for 13 weeks. The changes observed in the other organ-to-body weight ratios probably reflected the slower weight gain of the 4.0 ppm exposure group, rather than a direct

effect of acrolein exposure.

These investigations confirmed that the respiratory tract was the target organ system of inhaled acrolein. Although the insult delivered by exposure to 4.0 ppm resulted in pulmonary injury severe enough to be 56% fatal, extra-pulmonary organs were unaffected. Acrolein exposure had no apparent effect on bone marrow and peripheral blood lymphocyte populations. Also, the reproductive potential of exposed animals was unimpaired.

The pattern of histological change in the respiratory tract of animals in this study was similar to those previously reported (32,34). In one of the studies (32) marked pathology was observed in the nasal cavity of Wistar rats exposed to 0.4, 1.4, and 4.9 ppm acrolein for 13 weeks. Similarly, the nasal turbinates of the animals in this study showed an apparent dose dependent increase in submucosal lymphoid aggregates. However, rhinitis was only occasionally observed among the high dose animals in this study.

Contrary to the reports of acrolein toxicity for pulmonary macrophages (35,36), increased numbers were found in the bronchiolar regions of the 4.0 ppm animals. However, these cells may have accumulated in the damaged bronchioles during the post-exposure period.

The absence of overt pathologic changes in several of the animals from the 4.0 ppm exposure group was unexpected considering this acrolein concentration proved lethal to 56% of the male rats exposed. This marked intra-group variability was also evident in the 1.4 ppm group. Reasons for the observed intra-group variability are unclear, but genetic heterogeneity may have been responsible.

Follow-up histopathological examination was conducted on the exposed male rats used in the reproductive studies. These animals were necropsied after being maintained under non-SPF conditions for 10 weeks post-exposure.

A histopathology report on the findings in these animals has been provided in Appendix I. In brief, marked changes from control histology occurred only in the 4.0 ppm exposure group. A clustering of foamy intraalveolar macrophages attended by mononuclear intraseptal hypercellularity, both changes resembling low grade interstitial pneumonitis, were evident. Low grade subacute bronchitis was also observed. These changes were not considered specific to acrolein exposure; however, the limitation of these findings to the high dose group suggested an obvious association. Exposure to 4.0 ppm acrolein may have significantly suppressed the intrapulmonary killing of naturally occurring pathogenic entities. Acrolein exposure has been reported to interfere with pulmonary antibacterial and antiviral defenses (35,36). Bouley et al. (35) found that rats exposed to 0.55 ppm acrolein for 18 days were more susceptible to airborne Salmonella centeritidis than air exposed controls. However, when rats were exposed for 63 days and then infected, the death rates were identical for control and acrolein exposed animals. Although the susceptibility of rats to pathologic agents after exposure to higher concentrations has not been explored, it appears that exposure to 4.0 ppm may compromise the defense mechanisms of these animals for extended periods after exposure has been terminated.

Within the control group the correlation of elastin with FRC_b, lung weight with $\dot{V}_E$, and protein with PEF, EFR_{50}, and $\dot{V}_E$ simply indicated that large normal lungs have greater volumes and therefore greater maximum flows than smaller normal lungs. These associations are worthy of mention because they were not maintained in any of the acrolein exposure groups. The pathology ranking, which in the control group probably reflected occasional low level infective pneumonitis, was also significantly associated with the maximum flow of MEFV. However, no comfortable explanation for this association can be offered.

A substantial decrement in pulmonary function was observed in animals exposed to 4.0 ppm acrolein. The depressed flow-volume effort, the leftward shift of the quasi-static compliance curve, and the enlarged lung volumes suggested obstructive airway lesions. This overall functional impairment was consistent with the marked increase in connective tissue and the histological damage observed in this group. The increased connective tissue concentration, particularly elastin, was significantly associated with the distortion observed in the effort-independent limb of the MEFV curve (ΔEFR_{25}) and with the loss of maximum flow (EFR_{25}). The depression of the flow-volume curve (ΔEFR_{25}) in this group was also significantly correlated with the ranked scores of total lung injury. A significant association was also found between upstream airway resistance and elastin concentration. Presumably, the bronchiolar epithelial lesion observed in the 4.0 ppm group resulted in local connective tissue proliferation which accounted, in part, for the overall increased collagen and elastin concentrations.

Parenchymal damage attributable to 4.0 ppm acrolein was confined to the peribronchiolar regions. Although scattered macrophage accumulations accompanied foci of edema, these lesions were apparently too disperse and localized to reduce functional compliance, an indicator of gross restriction. In fact, before normalization to the corresponding increase in vital capacity, raw static compliance (QSC slope) was significantly elevated. This phenomena has been reported in growing animals, where unadjusted lung compliance increased with lung volume (37). However, after acrolein exposure the reduced specific compliance (QSC slope/FRC_d), which represented the lung at a relaxed end-tidal volume, did indicate significant restriction. Therefore, a proportionately greater fraction of the independent regions

of the deep lung may have been significantly injured, which resulted in the markedly lower specific compliance. The 337% increase in trapped air volume, 100% when expressed as a function of TLC_d, indicated that these rats were typically breathing at high lung volume (FRC_d or end-tidal volume). Because compliance of the normal lung falls with increasing volume, adjustment of the FRC by the trapped air volume would shift normal tidal breathing down the QSC curve. This would bring the compliance values of the 4.0 ppm animals into the normal range. A similar adjustment would also account for the apparent fall in C_{DYN}/FRC_d.

The expeditious washout of N_2 in the 4.0 ppm rats reflected tissue changes which increased the time constants of air turnover within the lungs. Stiffening of the airways and parenchyma, perhaps in conjunction with a reduction in lung compartmentalization, could account for these observations. The increased connective tissue concentrations in this exposure group would also support these observations; however, no generalized lesion suggestive of either interstitial or focal fibrosis was remarkable. The accelerated air turnover within the lung may have provided the ventilatory advantage to the undamaged lung needed to maintain adequate gas exchange. Unfortunately, blood gas determinations were not made in this study. However, diffusion of CO was elevated in these animals, probably as a function of lung volume, as it again fell within the control range when normalized to TLC.

The degree to which compensatory growth of injured lung may have biased the assessment of pulmonary function, particularly in the 4.0 ppm group, must be considered. Replacement of lung tissue (hyperplasia) and hypertrophy of lung cells after pneumonectomy in young animals lacking a fixed mediastinum has been reported (38). Compensatory growth capability wanes during the final stages of growth; development and maturation of the rat lung is complete

at approximately 14 weeks of age. The animals used here were approximately 13 weeks old and in the final stages of lung development when first exposed to acrolein. Although the phenomena of compensatory lung growth has not been reported in lungs acutely damaged by toxic agents, it was certainly suggested by the data reported here. In spite of enlarged lung volume (TLC), the expression of volume per gram of tissue virtually eliminated any differences from control values. Normalization of the QSC curves to VC (functioning lung) yielded compliance curves typical of controls. The DLCO as well as normalized DLCO (TLC) suggested increased lung tissue which apparently functioned normally. Finally, the increases observed in total connective tissue, protein, and DNA content were reduced when the increased lung size was taken into account. The fact that the ratios among these constituents did not change across exposure groups indicated a change in the amount, but not the composition of lung tissue in the 4.0 ppm group.

The reality of biological variability certainly presented itself in this study, particularly in the 4.0 ppm exposure group. While 56% of the male rats died during the exposure regime, only 6 of the 9 animals assessed physiologically exhibited overt histological damage, and only 7 of the 9 demonstrated functional changes distinct from the controls. The two animals with the least functional damage were also free of histologic injury. The third animal with unremarkable histologic change exhibited only minor functional deficit.

Exposure to 0.4 ppm acrolein resulted in airway changes which suggested greater rigidity or stability of the small airways. All flows, particularly those in the effort independent region of the forced expiratory curve, were significantly elevated relative to control values. The lower than control R_{us}, which reflected airway patency, may have allowed unusually high air

flow at low lung volume. These "supra-normal" flows were not affected by adjustment to lung volume. The slight rightward shift of the QSC curve indicated a mild restriction, possibly the result of parenchymal stiffening. Such rigidity would result in augmented flow dynamics. However, connective tissue changes did not correlate with the flow or compliance changes observed in this exposure group, and acrolein associated pathology was not apparent.

Without data from the 0.4 and 4.0 ppm groups, the functional response of the animals exposed to 1.4 ppm would, at best, have been uninterpretable, and at worst, misleading. Overall, the animals in this group did not differ functionally from the controls. Other than the slight but significant elevation in DLCO/TLC, only suggestive functional differences were evident. With the exception of the hydroxyproline concentration, none of the lung constituents changed from control values. However, the ranked pathology scores of the animals in this group were significantly associated with their elastin as well as hydroxyproline concentrations.

Pulmonary function tests are limited in that they describe the overall function of a complex system which possesses tremendous compensatory capability. Therefore, similar lesions may have different functional effects, depending on their location in the respiratory tree. Conversely, different lesions may result in similar functional changes, such as direct obstruction of small airways versus airway flaccidity. Therefore, lesions which differ in character, quality, or location may each independently or interdependently alter function. Such summation over the entire respiratory system produces a composite functional picture. Under the exposure regime studied, acrolein may have produced distinct lesions which expressed themselves in a contradictory or compensatory manner. The extremes of

the functional effects were observed at 0.4 and 4.0 ppm, while these effects essentially cancelled in the 1.4 ppm group and resulted in an apparently normal intermediate response.

Those variables from the array of data available which most effectively separated the exposure groups were selected by stepwise discriminant analysis. Among the lung composition parameters hydroxyproline concentration consistently appeared as a discriminating variable. However, DNA frequently added additional discriminating power to the lung composition variables. Among the pulmonary function variables, $DLCO_{sb}$, EFR_{25}, FRC_b, and R, proved to be the most discriminating. Considering the individual variability in response to acrolein exposure discussed earlier, the classification functions performed adequately. The only exception noted was in the 1.4 ppm group which could not be distinguished from controls on the basis of pulmonary function.

Many of the above mentioned variables and their associated discriminating power may be useful indicators of pulmonary health after exposure to toxic agents. On the other hand, these discriminating variables may be peculiar to acrolein exposed animals. The possibility that a limited number of variables may surface as discriminating when stepwise discriminant analysis has been applied to data from studies involving a variety of agents and animals species should not be overlooked. Should this be the case, testing regimes which include pulmonary function and/or composition assessment, could concentrate on those variables which frequently surface as discriminators.

The functional tests, conducted on rats after exposure to acrolein, were a more sensitive indicator of subtle pulmonary changes induced by this compound than was light microscopic histopathology. The imposed MEFV maneuver was most sensitive and indicated significant small airway damage

at all exposure levels. Connective tissue composition was also more indicative of low level irritant exposure than was histopathologic examination, although it was less sensitive than functional assessment. However, the lung composition data strongly supported the functional observations. The rather conventional instrumentation required for these biochemical assessments make this information more widely available among inhalation laboratories than the pulmonary function data. Although the functional battery provided the best information concerning the pulmonary health of exposed animals, placement of animals in their appropriate exposure groups was most successful when the compositional and functional data were combined.

Similar relative sensitivities of these investigative approaches were also observed with ozone (39). Analogous studies using these approaches will be conducted on a variety of toxic agents to determine whether this relative sensitivity is peculiar to certain classes of agents or if it is a generalized phenomena. Also, the fact that functional changes occur without detectable structural changes at the light microscopy level leads to the yet unanswered question; are there ultrastructure abnormalities in the lungs of animals showing subtle functional changes?

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APPENDIX A

ACROLEIN: CHEMICAL AND PHYSICAL INFORMATION

ACROLEIN

Chemical Abstract Services Registry Number: 107-02-8

Chemical Abstract Name: 2-Propenal

Other synonyms: acraldehyde; acrylic aldehyde; allyl aldehyde; propenal; prop-2-en-1-al; 2-propen-1-one

Chemical structure: $CH_2 = CH_2 - C\overset{O}{\underset{H}{}}$

Molecular weight: 56.06

Boiling point: 52.5°C (a)

Melting point: -87.7°C (a)

Density: d_4^{20} 4 0.8410 (a)

Solubility: Soluble in water, ethanol, ether, and acetone (a)

Volatility: Vapor pressure at 17.5°C is 200 mm (b)

Stability: Flash point, -26.1°C (c); polymerizes spontaneously, particularly in the presence of light, alkali, or strong acid (d)

Conversion factor in air: 1 ppm = 2.3 mg/m^3

Acrolein: Air Pollution Information

Threshold limit value: USA: 0.1 ppm (0.25 mg/m^3) 1974 (e)

Threshold odor concentration: Population Identification $Threshold_{50\%}$: 0.1 ppm (f)

Population Identification $Threshold_{100\%}$: 0.21 ppm (f)

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APPENDIX B

CHEMICAL METHOD FOR ANALYSIS OF CHAMBER ACROLEIN CONCENTRATION

From: "Methods of Air Sampling and Analysis," 2nd Edition, pp. 300-303. M. Katz, ed., American Public Health Association, Washington, D.C.

115.

Tentative Method of Analysis for Low Molecular Weight Aliphatic Aldehydes in the Atmosphere

43501-01-71T

1. Principle

1.1 Formaldehyde, acrolein and low molecular weight aldehydes are collected in 1% $NaHSO_3$ solution in midget impingers. Formaldehyde is measured in an aliquot of the collection medium by the chromotropic acid procedure, acrolein by a modified mercuric-chloride-hexylresorcinol procedure, and C_2–C_5 aldehydes by a gas chromatographic procedure. The method permits the analysis of all C_1–C_5 aldehydes in a sample (1).

The sampling procedure is not applicable for the determination of alcohols, esters or ketones in atmospheric samples, since bisulfite does not efficiently collect these materials. However, should some of these compounds be present in the atmosphere, their presence may be indicated by the appearance of peaks corresponding to their retention times in the chromatograms. The retention times for several of these compounds are shown along with the aldehydes in Table 115:1.

2. Range and Sensitivity

2.1 At sampling rates of 2 l/min over a 1 hr period, the following minimum concentrations can be determined:

CH_2O:	0.02 ppm
CH_3CHO:	0.02 ppm
CH_3CH_2CHO:	0.03 ppm
$(CH_3)_2CHCHO$:	0.03 ppm
$CH_2 = CHCHO$:	0.01 ppm

Shorter sampling periods are permissible for higher concentrations.

3. Interferences

3.1 Formaldehyde.

3.1.1 The chromotropic acid procedure has very little interference from other aldehydes. Saturated aldehydes give less than 0.01% positive interference, and the unsaturated aldehyde acrolein results in a few per cent positive interference. Ethanol and higher molecular weight alcohols and olefins in mixtures with formaldehyde are negative interferences. However, concentrations of alcohols in air are usually much lower than formaldehyde concentrations and, therefore, are not a serious interference.

3.1.2 Phenols result in a 10 to 20% negative interference when present at an 8:1 excess over formaldehyde. They are, however, ordinarily present in the atmosphere at lesser concentrations than formaldehyde and, therefore, are not a serious interference.

3.1.3 Ethylene and propylene in a 10:1 excess over formaldehyde result in a

5 to 10% negative interference and 2-methyl-1, 3-butadiene in a 15:1 excess over formaldehyde showed a 15% negative interference. Aromatic hydrocarbons also constitute a negative interference. It has recently been found that cyclohexanone causes a bleaching of the final color.

3.2 ACROLEIN.

3.2.1 There is no interference in the acrolein determination from ordinary quantities of sulfur dioxide, nitrogen dioxide, ozone and most organic air pollutants. A slight interference occurs from dienes: 1.5% for 1,3-butadiene and 2% for 1,3-pentadiene. The red color produced by some other aldehydes and undetermined materials does not interfere in spectrophotometric measurement.

4. Precision and Accuracy

4.1 Known standards can be determined to within ± 5% of the true value.

Table 115:I. Retention Times for Aldehydes, Ketones, Alcohols and Esters*

Compound	Time, Retention minutes
Acetaldehyde	3.5
Propionaldehyde	4.6
Acetone	5.1
Isobutylraldehyde	5.5
Methyl alcohol	6.1
Ethyl alcohol	6.7
Isopropyl alcohol	6.7
Ethyl acetate	7.0
n-Butyraldehyde	7.1
Methyl-ethyl ketone	7.7
Isopentanal	12.0
Crotonaldehyde	14.0

*Flow rate, temperature and conditions described in text.

No data are available on precision and accuracy for atmospheric samples.

5. Apparatus

5.1 ABSORBERS. All glass standard midget impingers are acceptable. A train of 2 bubblers in series is used.

5.2 AIR PUMP. A pump capable of drawing at least 2 l of air/min for 60 min through the sampling train is required.

5.3 AIR METERING DEVICE. Either a limiting orifice of approximately 2 l/min capacity or a glass flow meter can be used. Cleaning and frequent calibration are required if a limiting orifice is used.

5.4 SPECTROPHOTOMETER. This instrument should be capable of measuring the developed colors at 605 nm and 580 nm. The absorption bands are rather narrow, and thus a lower absorptivity may be expected in a broad-band instrument.

5.5 GAS CHROMATOGRAPH, with hydrogen flame detector and injection port sleeve (Varian 1200 or equivalent).

5.6 BOILING WATER BATH.

6. Reagents

6.1 DETERMINATION OF FORMALDEHYDE.

6.1.2 *Sodium formaldehyde bisulfite* (E.K. P6450)

6.1.3 *Chromotropic acid sodium salt, EK P230, 0.5% in water.* Filter just before using. Stable for one week if kept refrigerated.

6.1.4 *Sulfuric acid.* Conc reagent grade.

6.2 DETERMINATION OF ACROLEIN.

6.2.1 *$HgCl_2$-4-hexylresorcinol.* 0.30 g $HgCl_2$ and 2.5 g 4 hexylresorcinol are dissolved in 50 ml 95% ethanol. (Stable at least 3 weeks if kept refrigerated.)

6.2.2 *TCAA.* To a 1 lb bottle of trichloracetic acid add 23 ml distilled water and 25 ml 95% ethanol. Mix until all the TCAA has dissolved.

6.3 COLLECTION MEDIUM. Sodium bisulfite, 1% in water.

7. Procedure

7.1 COLLECTION OF SAMPLES. Two midget impingers, each containing 10 ml of 1% $NaHSO_3$ are connected in series with Tygon tubing. These are followed by and connected to an empty impinger (for meter protection) and a dry test meter and a source of suction. During sampling the impingers are immersed in an ice bath. Sampling rate of 2 l/min should be maintained.

Sampling duration will depend on the concentration of aldehydes in the air. One hr sampling time at 2 l/min is adequate for ambient concentrations.

After sampling is complete, the impingers are disconnected from the train, the inlet and outlet tubes are capped, and the impingers stored in an ice bath or at 6 C in a refrigerator until analyses are performed. Cold storage is necessary only if the acrolein determination cannot be performed within 4 hr of sampling.

7.2 ANALYSIS OF SAMPLES. (Each impinger is analyzed separately).

7.2.1 *Formaldehyde* **(1) (2).** Transfer a 2-ml aliquot of the absorbing solution to a 25-ml graduated tube. Add 0.2 ml chromotropic acid, and then, cautiously, 5.0 ml conc sulfuric acid. Mix well. Transfer to a boiling water bath and heat for 15 min. Cool the samples and add distilled water to the 10-ml mark. Cool, mix and transfer to a 16-mm cuvette, reading the transmittance at 580 nm. A blank containing 2 ml of 1% sodium bisulfite should be run along with the samples and used for 100% T setting. From a standard curve read μg of formaldehyde.

7.2.2 *Acrolein* **(1) (3).** To a 25-ml graduated tube add an aliquot of the collected sample in bisulfite containing no more than 30 μg acrolein. Add 1% sodium bisulfite (if necessary) to a volume of 4.0 ml. Add 1.0 ml of the $HgCl_2$-4-hexylresorcinol reagent and mix. Add 5.0 ml of TCAA reagent and mix again. Insert in a boiling water bath for 5 to 6 min, remove, and set aside until tubes reach room temperature. Centrifuge samples at 1500 rpm for 5 min to clear slight turbidity. One hr after heating, read in a spectrophotometer at 605 nm against a bisulfite blank prepared in the same fashion as the samples.

7.2.3 *C_2-C_5 Aldehydes* **(1).**

a. ANALYTICAL COLUMN 12' × ⅛" stainless steel packed with 15% w/w Carbowax 20 M on Chromosorb, 60 to 80 mesh, followed by 5' × ⅛" stainless steel Uncondinonylphthalate on firebrick, 100 to 200 mesh, prepared as follows: Ucon 50-HB-200, 1.5 g, and 1.4 g of dinonylphthalate are dissolved in chloroform and added to 13 g of firebrick. The solvent is evaporated at room temperature and the column packed in the usual manner.

b. Injection port sleeve: The inlet of the injection port contains a glass sleeve packed with solid Na_2CO_3. The Na_2CO_3 is held in place with glass wool plugs.

c. Conditions:

Injection port temperature, 160 to 170 C
Column temperature, 105 C
Detector temperature, 200 C
Nitrogen carrier gas flow rate, 14 ml/min
Hydrogen flow rate, 20 ml/min
Combustion air flow rate, 400/min

d. Procedure: A 4 μl sample of the bisulfite collection solution is injected into the packed sleeve at the injection port and the chromatogram is recorded. Table 114:I shows the relative retention times for a series of aldehydes and ketones in the C_2-C_5 range.

8. Calibration

8.1 FORMALDEHYDE.

8.1.1 *Preparation of standard curve.* To a 1-l volumetric flask add 0.4466 g sodium formaldehyde bisulfite and dilute to volume. This solution contains 0.1 mg formaldehyde per ml. Dilute to obtain standard solutions containing 1, 3, 5 and 7 μg formaldehyde per ml. Treat 2-ml aliquots as described in the procedure for color development. Read each at 580 nm after setting instrument at 100% T with the blank. Using semilog paper, graph the respective concentrations *vs.* transmittance.

8.2 ACROLEIN.

8.2.1 *Preparation of standard curve.* To 250 ml of 1% sodium bisulfite add 4.0 μl freshly distilled acrolein. This yields a standard containing 13.4 μg/ml. To a series of tubes add 0.5, 1.0, 1.5, and 2.0 ml of standard. Adjust the volumes to 4.0 ml with 1% bisulfite and develop color as described above. Plot data on semi-log paper.

8.3 C_2—C_5 ALDEHYDES.

8.3.1 *Calibration.* A mixed standard of C_2–C_5 aldehydes and ketones is prepared as follows:

a. Acetaldehyde-bisulfite solution: 0.336 g $CH_3CHO \cdot NaHSO_3$ (EK 791) is dissolved in 1 l of 1% $NaHSO_3$. This gives

a solution containing 100 μg/ml acetaldehyde.

b. To 10.0 ml of the above solution are added 40.0 ml of 1% $NaHSO_3$, and 8 μl of a mixture of equal volumes of propanal, isobutanal, butanal, isopentanal, pentanal crotonaldehyde, acetone and butanone.

The final solution contains 20 μg/ml acetaldehyde and 0.02 μl of each of the C_2–C_5 aldehydes and ketones per ml. Four μl of the standard are injected into the glass sleeve in the injection port of the chromatograph as described in the procedure, and the chromatogram is recorded.

9. Calculations

(1.23 μg formaldehyde = 1 μl (vol) at 25 C and 760 Torr)

9.1 FORMALDEHYDE. ppm formaldehyde (CH_2O) =

$$\frac{\text{total micrograms of } CH_2O \text{ in sample}}{1.23 \times \text{sample volume in liters}}$$

9.2 ACROLEIN.
(2.3 μg acrolein = 1.0 μl (vol) acrolein)

$$\text{ppm} = \frac{\text{total } \mu\text{g of acrolein in sample}}{2.3 \times \text{sample volume in liters}}$$

9.3 ALDEHYDES. Calculation of unknown sample concentration is made on the basis of comparative peak heights between standards and unknowns.

10. Effect of Storage

10.1 After sampling is complete, collection media are stored in an ice bath or refrigerator at 6 C. Cold storage is necessary only if acrolein is to be determined. Under cold storage conditions, analyses can be performed within 48 hr with no deterioration of collected samples.

11. References

1. LEVAGGI, D.A., and M. FELDSTEIN. 1970. The Determination of Formaldehyde, Acrolein and Low Molecular Weight Aldehydes in Industrial Emissions on a Single Collected Sample. *JAPCA*, 20:312.
2. AMERICAN PUBLIC HEALTH ASSOCIATION. 1977. Methods of Air Sampling and Analysis. 2nd ed. p. 297. Washington, D.C.
3. Ibid. p. 297.

Subcommittee 4
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F. A. MILLER
E. R STEPHENS
N. G. WHITE

APPENDIX C

LIST OF EXPOSURE DAYS ON WHICH WET CHEMICAL DETERMINATIONS OF CHAMBER CONCENTRATIONS WERE CONDUCTED

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APPENDIX D

PHOTOCOPIES OF CHAMBER DATA SHEETS FOR FOUR RANDOMLY SELECTED EXPOSURE DAYS

NEW TANK 1007 (45 lbs)

Day 11

ACROLEIN EXPOSURE -- CHAMBER DATA SHEET

Time Generator On 0900 Time Off 1500 Chamber Operator B. MASTON Date 16 June

Acrolein Tank at 150 lbs at start, 250 lbs at finish

Chamber I -- CONTROL

Magnehelic Static ____ Orifice ____

Time	PPM	Temp
0933	.0023	22.5
1031	.0007	22.0
1127	.0006	22.1
1227	.00036	22.5
1327	.0002	
1423	.00042	21.7

hrs X PPM (TWA) = CT

Chamber II -- LOW DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0958	0.387	87		21.6
1058	0.327	87↑	89↓	21.7
1132	0.456	87		21.8
1232	0.298	87↑	89	21.7
1332	0.397	90		
1328	0.387	90		21.7

6 hrs X 0.376 PPM (TWA) = CT 2.252

Chamber III -- INTERMEDIATE DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0943	1.398	77		21.6
1043	1.448	77↓	75	21.7
1137	1.279	75↑	77	21.8
1237	1.299	77↑	79	21.7
1337	1.482	79↓	77	
	1.378	77		21.7

6 hrs X 1.380 PPM (TWA) = CT 8.284

Chamber IV -- HIGH DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0948	3.589	72		21.4
1048	3.570	72		21.5
1142	3.411	72↑	74	21.5
1242	3.421	74		21.5
1342	3.887	74		
	3.788			21.5

6 hrs X 3.611 PPM (TWA) = CT 21.666

Relative Humidity: ____

Comments:

Day 27

ACROLEIN EXPOSURE -- CHAMBER DATA SHEET

Time Generator On 0850 Time Off 1450 Chamber Operator B Maston Date 8 July

Acrolein Tank at 1150 lbs at start, 350 lbs at finish

Chamber I -- CONTROL

Magnehelic Static____ Orifice____

Time	PPM	Temp
0931	0.00048	22.6
1031	0.00072	22.5
1131	0.00022	22.3
1231	0.00012	22.3
1349	-0.00004	22.2
1425	-0.00032	22.3

hrs X PPM (TWA) = CT

Chamber II -- LOW DOSE

Magnehelic Static____ Orifice____

Time	PPM	Rotometer		Temp
0936	0.398	87		22.2
1036	0.283	87↑	90	22.0
1136	0.694	94	87	22.1
1236	0.588	87		22.2
1352	0.408	87		21.9
1430	0.532	87		22.2

6 hrs X 0.482 PPM (TWA) = CT 2.892

Chamber III -- INTERMEDIATE DOSE

Magnehelic Static____ Orifice____

Time	PPM	Rotometer		Temp
0941	1.392	70		21.8
1041	1.392	70		21.8
1141	1.564	70		21.7
1241	1.487	70		21.7
1357	1.344	70		21.8
1435	1.449	70		21.9

6 hrs X 1.438 PPM (TWA) = CT 8.628

Chamber IV -- HIGH DOSE

Magnehelic Static____ Orifice____

Time	PPM	Rotometer		Temp
0946	3.943	75		21.3
1046	3.800	75		21.0
1146	4.230	75		21.0
1246	4.048	75		20.6
1402	3.723	75		20.8
1440	3.866	75		20.0

6 hrs X 3.935 PPM (TWA) = CT 23.61

Relative Humidity:____

Comments:

APPENDIX E

PULMONARY FUNCTION DATA FROM INDIVIDUAL FISCHER 344 RATS

Day 5-8

ACROLEIN EXPOSURE -- CHAMBER DATA SHEET

Time Generator On 0810 Time Off 1410 Chamber Operator RS. Master Date 20 Aug

Acrolein Tank at 1100 lbs at start, 250 lbs at finish

Chamber I -- CONTROL

Magnehelic Static ____ Orifice ____

Time	PPM	Temp
0855	0.0001	20.8
0955	0.0003	21.4
1055	0.00031	21.3
1150	-0.00006	21.2
1250	0.0001	21.5
1345	-0.00003	21.3

hrs X PPM (TWA) = CT

Chamber II -- LOW DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0955	0.299	80↑	82	20.7
1800	0.438	82		20.9
1105	0.327	82		21.1
1155	0.389	82		21.2
1255	0.363	82↑	84	21.2
1352	0.422	84		21.1

6 hrs X 0.373 PPM (TWA) = CT 2.230

Chamber III -- INTERMEDIATE DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0905	1.539	69↓	67	20.6
1005	1.465	67↓	65	20.6
1105	1.387	65		20.9
1200	1.482	65		21.0
1300	1.424	65		21.2
1355	1.359	65		20.9

6 hrs X 1.442 PPM (TWA) = CT 8.656

Chamber IV -- HIGH DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0910	3.927	72		19.4
1010	3.935	72		19.6
1110	3.857	72		19.6
1205	3.846	72		19.6
1305	3.813	72↑	74	19.7
1400	3.992	74		19.7

6 hrs X 3.895 PPM (TWA) = CT 23.37

Relative Humidity: ____

Comments:

Day 85

ACROLEIN EXPOSURE — CHAMBER DATA SHEET

Time Generator On 0905 Time Off 1405 Chamber Operator B. Hooton Date 26 Sept

Acrolein Tank at 700 lbs at start 1700 lbs at finish

Chamber I — CONTROL

Magnehelic Static ____ Orifice ____

Time	PPM	Temp
0940	0.0002	20.2
1030	0.00017	20.3
1130	0.00034	20.5
1230	0.00032	20.5
1305	0.00023	20.5
1410	0.0000	20.5

hrs X PPM (TWA) = CT

Chamber II — LOW DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0945	0.466	77		19.8
1035	0.399	77		20.0
1135	0.312	77		20.0
1235	0.340	77		20.3
1307	0.374	77	*	20.3
1415	0.404	77	*	20.2
1307	WET	TEST		
	0.345	77		

6 hrs X 0.382 PPM (TWA) = CT 2.295

Chamber III — INTERMEDIATE DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0950	1.497	72		19.6
1040	1.448	72		19.7
1140	1.380	72		20.1
1240	1.414	72		20.7
1321	1.367	72	*	20.2
1420	1.354	72	*	20.1
	WET	TEST		
1321	1.351	72		

6 hrs X 1.41 PPM (TWA) = CT 8.46

Chamber IV — HIGH DOSE

Magnehelic Static ____ Orifice ____

Time	PPM	Rotometer		Temp
0955	4.043	74		19.3
1045	3.926	74		19.5
1145	3.914	74		19.7
1245	3.842	74	*	19.7
1328	3.738	74		19.7
1425	3.776	74	*	19.6
	WET	TEST		
1328	3.671	74		

6 hrs X 3.873 PPM (TWA) = CT 23.239

Relative Humidity: ____

Comments: * NEW TANK 1401 1335

* PPM during wet Test

CONTROL GROUP

An #	HR	V_T	f	V_E	ΔP_L	R_L	C_{DYN}	FRC_d	FRC_b	RV_d
5501	393	2,12	61	129,32	11,06	1,09	,16	3,63	2,46	1,46
5502	436	1,65	75	123,75	10,41	1,25	,23	3,34	1,84	,59
5503	453	1,59	68	108,12	6,77	1,05	,20	2,61	1,87	,87
5517	421	1,59	52	82,68	6,96	1,09	,22	2,94	2,01	1,26
5518	407	1,49	66	98,34	10,28	,02	,16	2,69	4,12	2,87
5519	381	1,43	116	165,88	9,51	,93	,13	2,53	3,48	2,23
5588	421	1,82	69	125,58	14,86	,42	,32	2,74	3,34	1,59
5542	369	1,72	59	101,48	6,73	,25	,26	2,77	4,44	2,19
5543	343	1,70	32	54,40	11,80	1,17	,20	2,66	2,48	,48
5544	300	1,99	57	113,43	7,72	,48	,27	2,84	2,37	,87
5598	0	2,06	54	111,24	7,36	,37	,34	2,34	2,73	1,48
5546	444	1,96	64	125,44	8,67	,19	,25	2,53	2,31	1,31
5559	324	1,99	97	193,03	8,78	,62	,29	3,11	2,15	,90
5548	480	1,93	76	146,68	8,22	,97	,25	2,95	2,15	1,53
5549	421	2,28	75	171,00	7,77	1,25	,26	3,72	1,53	1,03
5550	444	1,58	77	121,66	9,62	,70	,26	2,73	2,73	1,61
5551	414	1,58	80	126,40	4,50	,59	,40	3,00	2,21	1,33
5552	400	1,55	62	96,10	11,73	,56	,14	2,83	2,50	1,25
5553	375	1,65	61	100,65	6,40	,93	,28	3,82	1,71	,96
5554	364	1,96	61	120,78	13,14	1,25	,23	3,15	1,92	,42
5555	353	1,74	60	104,40	14,00	,65	,12	3,19	2,35	1,10
5556	369	1,67	84	140,28	5,79	,93	,23	3,71	2,29	1,41
5591	393	1,72	74	127,28	4,21	,65	,29	3,71	2,78	1,28
5558	353	2,34	55	128,70	0,00	,93	,17	3,80	2,98	1,73

Data missing because of technical difficulties are indicated by a 0.

CONTROL GROUP

An #	VC	TLC_d	$DLCO_{sb}$	QSC_{ss}	PEF	EFR_{50}	EFR_{25}	EFR_{10}	ΔEFR_{25}
5501	7,25	8,71	,243	,50	104,2	100,4	62,4	30,9	12,20
5502	7.00	7,59	,175	,60	96,5	83,7	51,5	32,2	9,65
5503	8,50	9,37	,219	1,11	108,1	96,5	45,0	16,1	-3,25
5517	8,75	10,01	,239	1,33	95,2	75,9	54,1	28,3	16,15
5518	9,25	12,12	,274	1,08	109,4	106,8	72,1	38,6	18,70
5519	8,50	10,73	,213	,71	126,1	113,3	56,6	18,0	-,05
5588	9,75	11,34	,224	,69	103,0	90,1	54,1	15,4	9,05
5542	9,75	11,94	,218	,63	94,0	82,4	50,5	31,5	9,30
5543	8,25	8,73	,198	1,17	94,1	75,1	49,7	16,7	12,15
5544	8,50	9,37	,202	,75	87,5	86,9	61,1	-29,6	17,65
5598	9,50	10,98	,123	1,25	112,0	88,2	41,2	11,6	-2,90
5546	8,25	9,56	,217	1,29	87,5	82,4	43,8	23,2	2,60
5559	9,13	10,03	,211	,60	82,4	78,5	32,2	10,3	-7,05
5548	8,87	10,40	,266	,66	115,3	86,2	47,6	27,0	4,50
5549	8,38	9,41	,209	,75	88,8	72,1	42,5	28,3	6,45
5550	8,75	10,36	,245	,70	103,0	90,1	61,8	31,5	16,75
5551	8,93	10,26	,259	0,00	110,7	108,1	79,8	48,9	25,75
5552	9,00	10,25	,233	,75	90,1	76,6	38,6	14,2	,30
5553	9,13	10,09	,254	,75	83,7	61,8	15,4	3,2	-15,50
5554	9,38	9,80	,275	,86	101,0	83,7	59,2	37,3	17,35
5555	8,63	9,73	,212	,74	106,8	95,2	43,8	19,3	-3,80
5556	9,38	10,79	,257	,78	90,1	64,4	27,0	3,9	-5,20
5591	9,38	10,66	,234	,61	100,6	97,8	63,1	37,3	14,20
5558	9,25	10,98	,251	,63	104,3	90,1	34,7	10,3	-10,35

LOW DOSE

An #	HR	V_T	f	V_E	ΔP_L	R_L	C_{DYN}	FRC_d	FRC_b	RV_d
5601	316	2,06	64	111,24	12,73	,53	,18	3,03	1,39	,89
5602	444	1,65	55	90,75	9,63	1,17	,17	3,03	4,20	2,45
5687	369	1,59	71	112,89	11,44	,50	,12	3,06	1,81	,56
5617	429	2,05	83	170,15	9,29	1,11	,20	2,51	3,72	2,47
5618	369	1,99	78	155,22	12,88	,93	,13	3,02	2,48	1,23
5619	453	1,63	64	104,32	8,84	,90	,15	2,77	2,62	1,12
5697	333	1,60	67	107,20	11,85	,31	,14	2,60	2,45	,45
5642	381	1,56	66	102,96	9,61	,93	,12	3,92	4,04	3,54
5643	387	1,65	68	112,20	5,03	,16	,36	2,68	1,71	,21
5644	316	1,76	61	107,36	4,88	1,25	,37	2,62	2,94	,94
5645	405	1,07	65	158,95	6,80	,72	,36	2,93	5,24	3,74
5646	300	2,27	82	186,14	5,64	,28	,31	2,76	2,58	1,33
5692	308	2,03	41	83,23	9,85	,62	,17	3,03	2,67	1,29
5649	471	1,93	101	194,93	5,70	,23	,25	2,95	1,94	,94
5650	400	1,61	59	94,99	5,67	,20	,28	3,01	2,80	1,30
5693	393	2,35	70	164,50	8,60	,78	,22	3,11	1,89	1,27
5652	490	1,86	71	132,06	9,31	,93	,19	3,12	2,98	1,36
5694	375	1,53	53	81,09	7,69	,55	,35	2,86	3,04	1,79
5695	414	2,12	87	184,44	13,46	,70	,26	3,46	2,74	1,54
5655	417	1,87	72	134,64	7,19	,28	,28	4,17	2,14	1,39
5656	397	2,06	76	156,56	4,99	,45	,48	3,53	2,63	1,38
5657	356	1,47	104	152,88	5,18	1,17	,18	3,36	2,27	1,08
5696	407	1,80	55	99,00	6,23	1,01	,24	2,74	2,64	1,39

LOW DOSE

An #	VC	TLC_d	$DLCO_{sb}$	QSC_{ss}	PEF	EFR_{50}	EFR_{25}	EFR_{10}	ΔEFR_{25}
5601	9,25	10,14	,285	1,00	119,7	115,8	69,5	30,9	11,60
5602	8,25	10,70	,260	,57	108,1	101,7	70,8	34,7	19,95
5687	9,50	10,06	,247	,80	117,1	104,9	69,5	38,6	17,05
5617	8,00	10,47	,248	,57	113,3	108,1	75,9	37,3	21,85
5618	9,00	10,23	,232	,70	106,2	103,0	73,4	45,0	21,90
5619	7,50	8,62	,194	,71	127,4	125,5	90,1	46,3	27,35
5697	7,96	8,41	,218	,81	104,2	100,4	63,1	39,9	12,90
5642	7,00	10,54	,275	,56	96,5	91,4	68,2	38,6	22,50
5643	8,50	8,71	,214	1,00	99,1	82,4	46,3	7,7	5,10
5644	7,00	7,94	,203	,69	101,7	99,1	74,6	37,3	25,05
5645	8,25	11,99	,299	1,00	97,8	92,7	64,4	29,6	18,05
5646	10,50	11,83	,272	,93	121,0	105,5	73,4	47,6	30,65
5692	7,63	8,92	,223	,71	104,9	101,7	66,9	28,3	16,05
5649	7,50	8,44	,215	,58	103,0	101,7	79,8	29,6	28,95
5650	8,38	9,68	,224	,56	88,8	79,8	35,4	15,4	-4,50
5693	8,50	9,77	,241	,75	99,7	90,1	39,9	18,0	-5,15
5652	9,25	10,61	,272	,67	100,4	91,4	57,3	29,6	11,60
5694	9,00	10,79	,258	,77	87,5	86,9	65,6	36,0	22,15
5695	9,20	10,74	,279	,63	83,7	82,4	66,9	23,2	25,70
5655	9,00	10,39	,253	,73	97,8	84,9	57,9	35,4	15,45
5656	9,00	10,38	,262	,63	101,0	95,2	69,5	47,0	21,90
5657	9,13	10,21	,225	,80	103,0	87,5	59,2	24,5	15,45
5696	8,25	9,64	,233	,67	113,3	106,8	63,1	38,6	9,70

INTERMEDIATE DOSE

An #	HR	V_T	f	V_E	ΔP_L	R_L	C_{DYN}	FRC_d	FRC_b	RV_d
5701	387	1,87	74	138,38	7,72	,86	,22	3,48	2,57	1,57
5702	466	1,73	86	148,78	9,42	,59	,19	2,94	3,42	1,42
5703	462	1,71	54	92,34	6,03	,22	,24	2,77	1,65	,40
5798	440	1,69	77	130,13	10,73	,69	,20	3,63	4,53	3,03
5785	414	1,69	57	96,33	9,77	,75	,16	3,03	3,24	2,24
5742	358	1,70	49	83,30	11,28	,22	,16	2,63	2,75	1,25
5743	285	1,77	64	113,28	6,64	,78	,28	2,39	1,99	,99
5744	250	1,98	98	194,04	9,08	,75	,18	4,45	5,48	1,73
5745	353	1,69	62	104,78	8,83	,53	,19	0,00	****	****
5746	368	1,61	71	114,31	4,83	,76	,18	2,76	2,70	1,95
5747	387	1,54	79	121,66	10,82	1,79	,12	3,04	1,98	1,38
5799	421	1,69	86	145,34	9,85	,39	,14	3,07	2,99	1,74
5749	404	1,78	59	105,02	6,89	,42	,28	2,61	2,52	1,52
5793	304	1,87	68	127,16	8,03	,47	,23	3,49	3,08	1,66
5751	480	1,86	27	50,22	13,53	,78	,11	2,86	1,77	1,00
5752	429	2,07	129	267,03	8,72	1,01	,31	3,29	2,48	1,11
5753	455	2,18	82	178,76	6,90	,20	,24	4,16	2,31	1,06
5754	417	1,62	83	134,46	4,83	1,25	,18	3,00	2,57	1,62
5755	410	1,91	90	171,90	5,56	1,71	,30	4,26	3,18	1,68
5756	421	1,71	57	97,47	5,94	,70	,17	3,35	2,62	1,22
5757	350	1,83	67	122,61	9,13	,47	,14	3,80	3,22	1,72
5758	397	1,81	56	101,36	5,78	1,25	,22	3,41	2,62	1,49

INTERMEDIATE DOSE

An #	VC	TLC_d	$DLCO_{sb}$	QSC_{ss}	PEF	EFR50	EFR_{25}	EFR_{10}	ΔEFR_{25}
5701	9,50	11,07	,210	,64	118,4	110,0	77,2	41,2	22,20
5702	10,00	11,42	,261	,86	103,0	92,7	38,6	25,7	-7,75
5703	9,75	10,15	,233	,75	100,4	84,9	56,6	21,2	14,15
5798	11,50	14,53	,335	1,25	115,8	108,1	74,6	38,6	20,55
5785	10,25	12,49	,281	,69	114,5	96,5	61,8	32,2	13,55
5742	8,25	9,50	,209	1,00	110,7	100,4	57,9	30,9	7,70
5743	9,00	9,99	,223	,92	110,7	97,8	67,6	28,3	18,70
5744	9,00	10,73	,401	,50	84,9	70,8	42,5	15,4	7,10
5745	9,00	0,00	0,000	,56	82,4	62,4	24,5	14,2	-6,70
5746	8,25	10,20	,243	,75	110,0	105,5	68,2	30,9	15,45
5747	7,75	9,13	,210	,61	94,0	92,7	69,5	36,0	23,15
5799	8,00	9,74	,232	,75	108,1	94,0	55,0	34,7	8,00
5749	9,50	11,02	,252	,81	100,4	100,4	69,5	14,2	19,30
5793	9,50	11,16	,265	,60	105,5	104,2	61,8	43,8	9,70
5751	9,40	10,40	,241	,77	115,8	106,8	64,4	19,3	11,00
5752	8,50	9,61	,233	,63	92,7	79,8	54,7	36,0	14,80
5753	9,25	10,31	,253	,97	112,6	108,1	77,2	55,3	23,15
5754	7,20	8,62	,242	,50	66,9	65,6	33,5	28,3	,70
5755	8,88	10,56	,260	1,00	-0,0	-0,0	0,0	0,0	0,00
5756	8,40	9,62	,240	,47	106,8	83,7	30,9	6,4	-10,95
5757	9,13	10,85	,279	1,30	74,6	65,6	12,9	2,6	-19,90
5758	9,10	10,59	,237	,67	101,7	99,7	36,0	3,2	-13,85

HIGH DOSE

An #	HR	V_T	f	V_E	ΔP_L	R_L	C_{DYN}	FRC_d	FRC_b	RV_d
5801	400	2,46	45	110,70	9,93	1,87	,28	7,20	3,13	2,88
5810	425	2,13	52	110,76	14,13	1,56	,13	4,16	4,22	2,72
5842	446	2,70	34	91,80	10,44	1,36	,20	7,86	3,83	2,58
5843	400	1,93	31	59,83	12,83	2,49	,17	8,77	6,56	4,76
5845	444	2,11	69	145,59	5,84	,56	,34	4,73	3,23	1,98
5859	414	2,38	21	49,98	16,22	,86	,17	9,86	9,86	6,57
5886	440	2,63	37	97,31	10,19	,74	,23	6,11	3,73	3,23
5889	375	1,91	33	63,03	13,70	1,01	,43	7,76	4,59	3,09
5890	333	2,16	38	82,08	5,47	2,03	,35	4,85	5,02	3,52

HIGH DOSE

An #	VC	TLC_d	$DLCO_{sb}$	QSC_{ss}	PEF	EFR_{50}	EFR_{25}	EFR_{10}	ΔEFR_{25}
5801	11,25	14,13	,300	,83	70,1	38,6	14,2	5,1	-5,10
5818	12,69	15,41	,333	1,25	104,2	97,8	61,8	12,9	12,90
5842	9,46	12,04	,280	,83	61,8	47,6	36,0	28,3	12,20
5843	11,30	16,06	,316	,83	77,2	47,6	10,3	5,8	-13,50
5845	8,82	10,80	,275	,67	92,7	82,4	47,6	7,1	6,40
5859	11,29	17,86	,527	,67	90,1	54,1	5,1	2,6	-21,95
5886	12,25	15,48	,335	1,44	86,9	52,8	21,9	5,1	-4,50
5889	13,00	16,09	,316	1,43	95,2	90,1	51,5	18,0	6,45
5890	11,00	14,52	,280	,67	87,5	74,6	25,7	2,6	-11,60

APPENDIX F

LUNG COMPOSITION DATA FOR INDIVIDUAL FISCHER 344 RATS

CONTROL (0.0 ppm ACROLEIN)

ANIMAL #		LUNG WEIGHT (gms)	DRY WEIGHT (mg/gm)	TOTAL PROTEIN (mg)	TOTAL DNA (mg)	TOTAL HYDROXYPROLINE (mg)	TOTAL ELASTIN (mg)
5501	1	1.1748	239.41[illegible]	137.3610	5.7316	2.4653	4.8361
5502	2	1.2870	267.66[illegible]	147.6900	6.1650	2.1960	5.2020
5503	3	1.1340	234.18[illegible]	134.1910	5.5980	2.1240	5.9220
5517	4	1.3632	292.9920	155.1360	6.6432	2.3904	5.7216
5516	5	1.2555	204.[illegible]27[illegible]	144.8010	6.3240	2.4273	6.3147
5519	6	1.6187	316.3160	165.3670	7.5985	2.4934	5.5601
5588	7	1.4626	302.2290	167.7160	6.9552	2.9164	5.4280
5542	8	1.1659	246.6190	132.7880	5.6248	2.1983	5.4468
5543	9	1.4345	307.7650	167.3900	7.2200	2.2990	5.6145
5544	10	1.4194	284.3500	155.3820	5.6938	2.5662	5.6316
5598	11	1.3164	269.9280	143.3360	6.1640	2.2632	5.4096
5546	12	1.3248	276.6000	146.9240	6.7344	2.3184	4.8208
5559	13	1.3113	250.3560	150.2680	6.0450	2.2134	6.3984
5548	14	1.1921	237.3280	134.7710	5.6420	2.3660	6.0242
5549	15	1.3164	262.7170	142.7790	6.1789	2.6026	6.1334
5550	16	1.2184	255.2520	142.9340	6.0342	2.2606	5.6960
5551	17	1.1960	266.2480	144.6240	6.0628	2.5116	6.2100
5552	18	1.0920	229.4110	123.6690	5.6966	2.2841	6.0424
5553	19	1.1837	244.2160	126.2820	5.9274	2.1627	6.2745
5554	20	1.4168	311.5120	172.4080	7.1288	2.8888	7.5992
5555	21	1.2376	249.6130	143.4160	6.0151	2.2386	5.9241
5556	22	1.3650	308.4980	155.1550	6.7158	2.2932	6.4155
5591	23	1.2631	239.9670	141.4140	5.5055	2.1840	6.1971
5558	24	1.2927	272.3970	145.7310	6.1845	2.4552	6.9564

0.4 ppm ACROLEIN

ANIMAL #		LUNG WEIGHT (gms)	DRY WEIGHT (mg/gm)	TOTAL PROTEIN (mg)	TOTAL DNA (mg)	TOTAL HYDROXYPROLINE (mg)	TOTAL ELASTIN (mg)
56[illegible]1	25	1.1960	239.2[illegible]	138.2760	5.7408	2.6220	5.6948
56[illegible]2	26	1.[illegible]375	231.[illegible]9[illegible]	127.6730	5.5874	2.1658	6.4155
5687	27	1.1961	244.[illegible]760	135.9760	5.5292	2.5484	5.4280
5617	28	1.4570	238.[illegible]160	166.5420	6.8902	2.7730	5.5554
5618	29	1.[illegible]35[illegible]	277.4[illegible]6	153.8050	6.4125	2.8690	5.6240
5619	30	1.[illegible]440	230.56[illegible]6	132.8470	5.3190	2.6010	5.5710
5697	31	1.5624	327.8250	167.1160	7.4679	2.6970	6.4449
5642	32	1.5675	321.[illegible]050	162.1150	7.5335	2.7455	6.5360
5643	33	1.4345	279.[illegible]700	148.3950	6.2225	2.3940	5.5670
5644	34	1.2462	251.7510	136.1520	5.7567	2.3994	5.7660
5645	35	1.3616	285.2920	157.3200	6.9828	2.2172	5.5384
5646	36	1.3559	316.9430	172.[illegible]090	6.7704	2.2295	5.2780
5692	37	1.2870	196.1100	144.5400	5.9760	2.3220	6.[illegible]030
5649	38	1.2512	253.6440	139.8460	5.8696	2.5852	6.3204
5651	39	1.2328	253.6220	136.1680	5.6304	2.6588	6.4492
5693	40	1.2972	265.1440	14[illegible].8520	5.9340	2.8152	6.7068
5652	41	1.1532	222.3630	119.1330	5.5360	2.2134	5.7195
5694	42	1.3536	279.3680	146.8020	6.9090	2.8482	6.8432
5695	43	1.[illegible]450	229.14[illegible]6	113.4650	5.3200	2.4890	5.4625
5655	44	1.3144	249.12[illegible]0	14[illegible].3100	5.9580	2.1510	5.9310
5656	45	1.3013	265.3560	145.9640	6.3518	2.4388	7.2345
5657	46	1.1375	238.[illegible]560	130.4940	5.9605	2.3569	6.4064
5696	47	1.334[illegible]	316.6640	163.[illegible]240	7.1208	3.0268	7.8476

1.4 ppm ACROLEIN

ANIMAL #		LUNG WEIGHT (gms)	DRY WEIGHT (mg/gm)	TOTAL PROTEIN (mg)	TOTAL DNA (mg)	TOTAL HYDROXYPROLINE (mg)	TOTAL ELASTIN (mg)
5701	48	1.1250	252.9100	126.5400	5.6610	2.5650	5.1390
5702	49	1.3559	275.9120	157.1570	6.5975	2.6754	6.2972
5703	50	1.2696	259.6240	156.0320	5.7500	2.9256	6.0076
5798	51	1.1645	229.8410	142.0350	5.2870	2.6350	5.5080
5785	52	1.1700	230.8500	142.2900	5.4540	2.7630	6.1290
5742	53	1.3559	278.0960	155.8835	6.5065	2.6208	5.6966
5743	54	1.3950	278.2800	153.9950	6.5430	2.5380	5.7420
5744	55	1.3442	278.6040	153.0140	6.2886	3.0174	7.3226
5745	56	1.6461	359.6310	202.1820	7.7283	3.1341	6.1287
5746	57	1.9000	396.6720	221.3200	7.1520	3.0720	5.8464
5747	58	1.2870	248.7610	137.9700	5.9850	2.5110	6.1650
5792	59	1.2788	294.7680	173.1440	6.7252	3.2292	8.1328
5749	60	1.2512	245.0580	133.4830	6.3920	2.6790	6.8150
5793	61	1.3140	252.2700	139.0500	6.0390	2.6379	5.9220
5751	62	1.3346	282.9460	149.9300	6.1100	3.2994	8.3284
5752	63	1.2684	231.3360	152.0760	6.0076	2.8796	6.9460
5753	64	1.3064	281.2440	149.8680	6.2744	3.1372	7.1300
5754	65	1.6910	298.4910	177.5550	6.0230	2.7075	6.1275
5755	66	1.3671	258.6330	145.3590	5.8032	2.4831	6.4170
5756	67	1.3578	271.6530	142.6620	6.8541	2.7528	6.2124
5757	68	1.2420	251.9880	134.7800	5.8604	2.6036	6.4032
5758	69	1.2328	265.7000	147.1080	5.8420	3.0544	6.9460

4.0 ppm ACROLEIN

ANIMAL #		LUNG WEIGHT (gms)	DRY WEIGHT (mg/gm)	TOTAL PROTEIN (mg)	TOTAL DNA (mg)	TOTAL HYDROXYPROLINE (mg)	TOTAL ELASTIN (mg)
58.1	7.	.6955	333.1456	172.5210	7.1630	3.5245	12.5115
5816	71	1.4664	271.7546	147.9560	6.5518	3.6378	8.6668
5842	72	1.6916	324.6050	179.7400	7.3720	3.5435	9.7850
5843	73	1.6048	MISSING	MISSING	MISSING	MISSING	MISSING
5845	74	1.6544	341.3140	195.7060	7.0976	3.8164	9.3436
5859	75	1.9095	323.0950	184.5850	7.3055	4.6455	15.5705
5886	76	1.6156	341.9059	176.3270	7.5335	4.3225	15.8175
5889	77	1.9926	367.9160	192.7000	7.8366	4.2206	13.8650
5890	78	1.6150	317.6850	172.2350	7.3815	4.1990	14.7060

APPENDIX G

ABNORMAL SPERM DATA FROM INDIVIDUAL RATS

Percent Abnormal Sperm from Fischer 344 Rats Exposed to Filtered Air, 0.4, 1.4, or 4.0 ppm Acrolein

Acrolein Concentration (ppm)	Animal Number		Raw Data	arcsin $\sqrt{p}$
0.0	5501		1.0	5.74
	5502		1.0	5.74
	5503		4.2	11.83
	5504		1.0	5.74
	5506		1.4	6.80
	5517		1.8	7.71
	5519		1.0	5.74
	5542		2.0	8.13
	5584		2.6	9.28
	5588		2.2	8.53
		mean(±s.e.)	1.8(0.36)	7.52(0.706)
0.4	5602		0.4	3.63
	5607		0.8	5.13
	5608		9.0	17.46
	5617		1.4	6.80
	5619		4.0	11.54
	5642		2.2	8.53
	5645		1.6	7.27
	5687		1.6	7.27
	5697		2.0	8.13
		mean(±s.e.)	2.6(0.98)	8.42(1.515)
1.4	5701		1.6	7.27
	5702		1.0	5.74
	5703		0.8	5.13
	5704		2.0	8.13
	5745		2.0	8.13
	5746		1.4	6.78
	5747		1.4	6.78
	5785		3.2	10.30
	5786		3.6	10.94
	5798		1.0	5.74
		mean(±s.e.)	1.8(0.33)	7.49(2.030)
4.0	5805		0.6	4.44
	5806		2.4	8.91
	5808		1.0	5.74
	5818		3.2	10.30
	5820		7.2	15.56
	5823		3.8	11.24
	5842		3.6	10.94
	5845		2.0	8.13
	5859		3.4	10.63
	5884		1.6	7.27
		mean(±s.e.)	2.9(0.66)	9.32(1.113)

APPENDIX H

CANONICAL ANALYSIS PLOTS OF PULMONARY DATA FROM FISCHER 344 RATS EXPOSED TO ACROLEIN

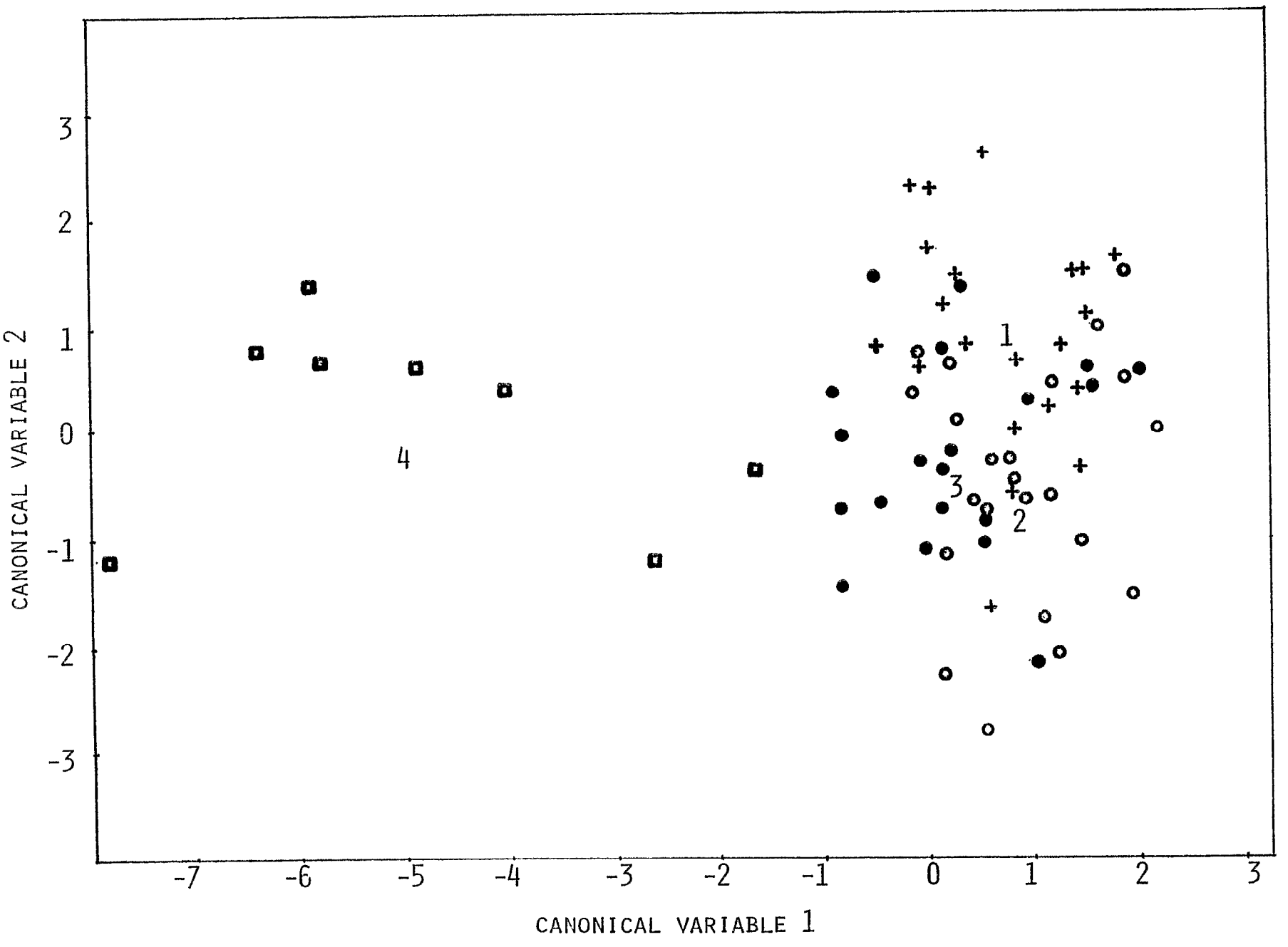
Canonical analysis plots of Fischer 344 rats exposed to either 0.0 (+), 0.4 (o), 1.4 (●), or 4.0 (▫) ppm acrolein, the centroids of each group are indicated by 1, 2, 3, or 4, respectively. The canonical variables are based on both respiratory physiology and lung composition parameters.

Page I-2

0.0, 0.4, 1.4, and 4.0 ppm exposure groups

Page I-3

0.0, 0.4, and 1.4 ppm exposure groups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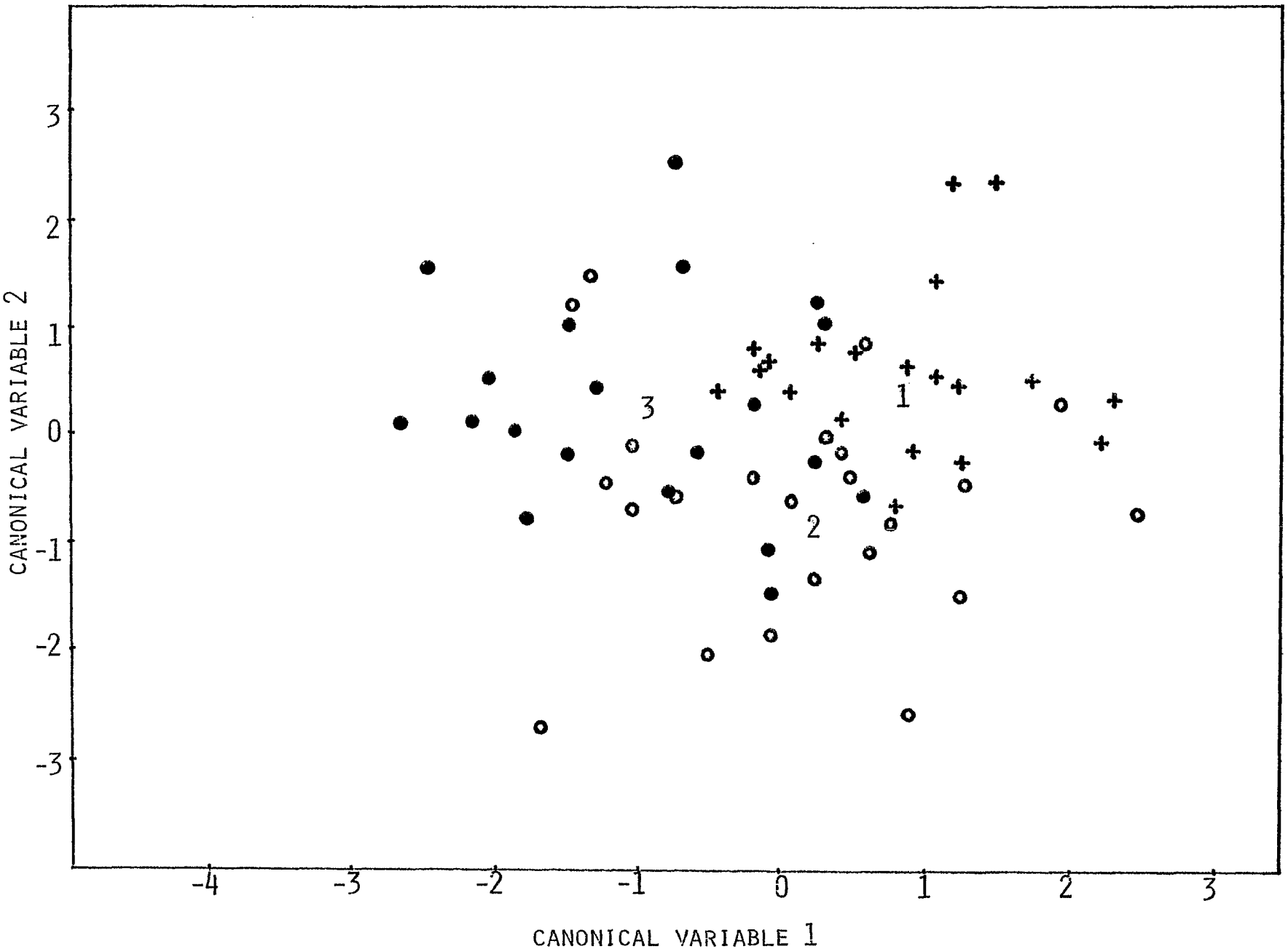
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CANONICAL VARIABLE 1
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2
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-1
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-3
-4
0
1
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3

APPENDIX I

FOLLOW-UP PULMONARY HISTOPATHOLOGY ON RATS MAINTAINED UNDER NON-SPF CONDITIONS FOR TEN WEEKS AFTER SIX DAY POST-EXPOSURE RECOVERY PERIOD

BROOKHAVEN NATIONAL LABORATORY

MEMORANDUM

DATE: March 13, 1981

TO: R. T. Drew and R. S. Kutzman

FROM: R. W. Wehner

SUBJECT: Pulmonary pathology of Fischer 344 rats following acrolein exposure and ten week maintenance on room air.

The attached report describes the findings of the microscopic examination of the exposed rats. The high dose group displayed changes associated with a wide range of insults, but little that could be directly related to the toxicant exposure. The role of acrolein in these animals may simply be one of aggrevating pre-existent pathologic entities.

The subtle morphologic alteration of cardiac anatomy is thought to represent processes associated with aging and spontaneous disease.

jc
Attachment

Control Animals (#5527, 5531, 5529, 5530, 5532, 5525)

H&E stained slides of transverse biventricular heart sections failed to disclose specific pathologic change. Neither myocardial fiber hypertrophy nor necrosis was recognized. Increased leukocytic (lymphocytic) populations between fibers seen in two of the six control rats were thought characteristic of low-grade chronic murine myocarditis. Similar lymphocytic hypercellularity, in two other heart sections, was uniformly perivascular in location and thought associated with natural aging processes.

Three of the six control lungs examined microscopically were normal. Two of six animals displayed small compact collections of sloughed airway epithelium within alveolar lumina. The pathophysiologic events leading to focal partial detachment of airway epithelium is unclear. Sections of lung from one animal displayed prominent lymphoid tissue, both the BALT and peripheral aggregates, possibly indicative of prior challenge. Medical calcification of medium sized arteries was additionally found in the same rat (#5530) and in all likelihood would represent a phenomenon associated with aging.

Low Dose Animals (0.4 ppm acrolein) (#5625, 5626, 5630, 5632).

Sections of cardiac ventricles were similar to control animals in appearance. The low grade chronic myocarditis was no more severe than that recognized in the control group.

Pulmonary parenchyma in these animals was essentially normal excepting modest lymphoid hyperplasia in two rats (5626, 5630) and small aggregates of sloughed epithelium. Neither of these changes can logically be solely attributed to the toxicant exposure.

Intermediate Dose Animals (1.4 ppm) (#5732, 5797, 5730, 5727, 5725).

Sections of heart were similar to control and low dose animals. Frank myocardial fiber hypertrophy, necrosis, or thrombosis was not recognized in any of the sections.

Likewise the pulmonary morphology was essentially normal. Although focal medial calcification, and small intraalveolar collections of sloughed epithelium were seen, changes considered toxicant related were not readily recognized.

High Dose Animals (4.0 ppm) (#5825, 5826, 5036, no tag).

Sections of heart from the rats exposed to the high dose of acrolein were similar in appearance to the other three groups, i.e. no toxicant specific lesion or tissue alteration was noted. The changes present, chronic murine myocarditis and perivascular lymphocytic hypercellularity were thought related to aging processes.

The sections of lung examined from this group of animals displayed a consistent pattern of pulmonary injury. The clustering of foamy, sometimes pigmented, intraalveolar macrophages was always attended by a mononuclear intraseptal hypercellularity, the two changes mutually resembling low grade interstitial pneumonitis. Moreover, this change was usually found in a subpleural location, that most often associated with chronic murine interstitial pneumonia. Inflammatory cells suspended in mucus within airway lumina were found in two animals and may well represent residual low grade subacute bronchitis. No proliferative epithelial changes were recognized in any of the sections. The presence of interstitial pneumonia, clustering of macrophages, and even mild subacute bronchitis are not specific injuries following the insult of acrolein inhalation. The limitation of these changes to the high dose group, however, suggests an obvious association with the toxicant exposure, possibly as an agitator of pre-existing, naturally occurring pathologic entities.